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THE PRODUCTION OF ARTIFICIAL IMMUNITY AGAINST TUBERCULOSIS IN CATTLE

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PREFACE

The work of the State Livestock Sanitary Board upon the immunization of animals against tuberculosis was begun in 1900, under a fund for special investigation of the diseases of animals. After the possibilities of this work were realized in 1903 a request was made to the Legislature for a larger fund and the purchase of a farm for the continuation of this and other researches in the field of animal diseases.

The State Livestock Sanitary Board at that time was composed of Hon. Samuel W. Pennypacker, Governor, Hon. N. B. Critchfield, Secretary of Agriculture, Hon. James Foust, Dairy and Food Commissioner and Dr. Leonard Pearson, State Veterinarian. To these men credit is due for obtaining the means by which it was possible to start these investigations. Upon the election of Hon. Edwin S Stuart as Governor in 1905, he became, by virtue of his office, the President of the Board, the other Members remaining unchanged until the death of Dr. Leonard Pearson, when the writer was appointed to succeed him as Secretary.

The work was well supported by all the Members of the Board. To a Committee consisting of the Treasurer, Hon. N. B. Critchfield and the Secretary, Dr. Leonard Pearson, was given the supervision of the farm, and to them much credit is due for the alteration and addition of buildings, making it possible to pursue the various phases of the work.

To Dr. Leonard Pearson we are indebted for the original plans of the experiments and through his able presentation of the importance of the subject to the Members of the Legislature it was possible to obtain the funds by which the work was continued. Difficulties arose in the progress of the work that seemed insurmountable but through the wise counsel, kindly suggestions and encouraging words of this one man, the parties in direct charge of the work were stimulated to exert their best efforts.

The writer is responsible for the laboratory work and management of the farm and care of the animals in all of the experiments excepting the last three, numbers 115M, 115N and 115O. The work of these experiments was done entirely by Drs. E. S. Deubler and John Reichel.

During the progress of the work valuable assistance was rendered by a number of men and to these men acknowledgement is made of their untiring efforts in behalf of this work: to Dr. M. P. Ravenel for the isolation of most of the cultures used in the preparation of the vaccine and for valuable assistance and advice; to Dr. C. Y. White and Dr. John Reichel for the histological examinations; to Drs. E. S. Deubler and John Reichel for the preparation of the vaccine for the latter experiments and general bacteriological work.

To Dr. E. S. Deubler credit is due for many of the autopsies, the vaccination of the animals and the management of the farm during the latter experiments. For general assistance rendered throughout the various phases of the work, acknowledgement is made to Drs. H. C. Campbell, the late A. A. Harmon, E. P. Althouse, E. Barnett and I. B. Powell.

By the efforts of our present Governor, Hon. John K. Tener, and the other members of the State Livestock Sanitary Board, means were devised by which it was possible to publish this work.

The writer feels deeply conscious of the fact that these experiments could have been presented in a more concise, intelligent and able manner by the one who was so instrumental in planning and starting them, but whose untimely death was such a severe loss to the entire scientific world and to the livestock interests of the Country.

PART I.

The work of the State Livestock Sanitary Board of Pennsylvania upon the Production of Artificial Immunity against Tuberculosis in Cattle. pg. 5.

PART II.

A review of the Production of Immunity in Cattle against Tuberculosis and the use of Tuberculin. pg. 83.

THE PRODUCTION OF ARTIFICIAL IMMUNITY AGAINST TUBERCULOSIS IN CATTLE

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(From the Laboratory of the State Livestock Sanitary Board of Pennsylvania)

The work of the State Livestock Sanitary Board of Pennsylvania upon the production of immunity against tuberculosis in cattle, began in September, 1900, when two animals were inoculated with large quantities of a culture of tubercle bacilli obtained from human sputum, with the idea of proving whether such cultures were capable of infection, and further, whether any immunity was obtained by the injection of doses that would not infect the animal. Systematic efforts to immunize animals against tuberculosis may be said to have commenced earnestly with the discovery of tuberculin by Koch in 1890. Progress in this line did not occur until efforts were made to immunize animals against tuberculosis by the use of living tubercle bacilli. It has been shown by numerous experiments that the sputum of persons suffering from consumption and cultures of tubercle bacilli made from such sputum are usually comparatively non-virulent for cattle. It is important to know further that a culture of tubercle bacilli is incapable of producing serious disease in such quantities as it may be necessary to use in an attempt to increase an animal's resistance to tuberculosis. A Jersey heifer, No. 26415, shown by the tuberculin test to be free from tuberculosis, was inoculated intraperitoneally September 29, 1900, with 4 cc. of a suspension of human sputum culture. This animal was inoculated about six months later with 13.5 cc. of a standard suspension of tubercle bacilli which had been recovered in pure culture by Dr. Theobald Smith in 1895 from a coati (Nasua Narica). Another intravenous inoculation with 10 cc. of a similar suspension was made three months later. On August 23, 1901, this heifer was inoculated with 20 cc. of a standard suspension in water of Culture M of tubercle bacilli from human sputum. This quantity of material was divided into four parts of 5 cc. each and these parts were injected beneath the skin into the peritoneal cavity, into the jugular vein and into the lung respectively. These injections were repeated at intervals of from seven to ten days until January 29, 1902. The quantity of standard suspension was increased 10 cc. with each successive inoculation so that at the last, the eighteenth inoculation, the total quantity given was 180 cc. The total quantity given in this series of inoculations was 1,797 cc. of a standard suspension. The first inoculation of the suspension of tubercle bacilli caused no temperature reaction. However, there was a rise of temperature from 2° to 4° following many of the later inoculations. The heifer was in a strong, thrifty condition at the completion of the series of inoculations on January 29, 1902, and improved in condition throughout the following months. It was killed on August 14, 1902. There was an abundance of fat upon the carcass and about the intestines. A very careful examination was made of all of the organs, membranes, etc. The lungs were normal in color and elastic; they were free from nodules but were attached to the chest walls along the lower borders by fibrous bands. A few flakes of fibrin were found upon the omentum and these flakes contained a few calcareous nodules about 1-12 of an inch in diameter. The liver was adherent to the diaphragm over an area 5 inches in diameter, but no part of the substance of the liver showed any evidence of tuberculosis.

A yearling grade short-horned bull No. 26442, after having been tested with tuberculin and proven to be free from tuberculosis, was inoculated intraperitoneally November 19, 1900, with 16 cc. of a suspension of tubercle bacilli of a culture from human sputum. On March 17, 1901, this animal was inoculated intravenously with 13.5 cc. of a suspension of a culture of tubercle bacilli similar to that used for the inoculation of the foregoing heifer, No. 26415, on March 15th. This bull was subsequently inoculated in the same manner as the heifer, receiving in all eighteen inoculations between August 23, 1901 and January 10, 1902. He received in all 1,710 cc. of a standard suspension of culture M of tubercle bacilli from human sputum. He reacted following the inoculations very much as the heifer, although somewhat more slowly. He remained in good condition throughout the entire experiment excepting for the development of an abscess over the jugular vein at the point of inoculations, which was opened and afterwards healed nicely. On January 18, 1902, eight days following the inoculation of the tubercle bacilli of the human type, this animal was inoculated intraperitoneally with 10 cc. of a standard suspension of tubercle bacilli of bovine origin known as Culture H. The virulence of this culture for cattle had been proven by numerous inoculations. It was found to be highly virulent. A cow weighing 950 lbs inoculated with 5 cc. of a standard suspension died in two months, and on post-mortem examination revealed a most extensive miliary tuberculosis of the lungs. A second cow weighing 698 lbs. was given two injections of 4-10 of a cc. each with an interval of one week. This animal died of miliary tuberculosis of the lungs. An eight month old heifer was inoculated intraperitoneally with 5 cc. of a standard suspension of this bovine culture and six weeks later died, and was found to contain extensive lesions of tuberculosis of the peritoneum and abdominal organs. The lungs were also studded with small tubercles. This bovine culture R was therefore considered highly virulent for cattle. The bull, No. 26442, was killed August 13, 1902, approximately seven months after it had received the injection of tubercle bacilli of bovine origin. The general condition of the animal was good and there was much fat upon the carcass and about the internal organs. The pleura lining the lower half of the chest was covered by a sheet of partly organized fibrin from 1-8 to 1-3 of an inch thick. The lungs themselves contained a few nodules about ½ inch in diameter, surrounded by thick walls and containing caseous pus in which there were many tubercle bacilli. These nodules did not seem to be progressive and appeared to be abscesses, indicating the sites of previous inoculations. The peritoneum covering the abdominal walls, the stomach, intestines, spleen and liver was coated with a layer of partly organized fibrin as in the chest wall. The lymphatic glands about the rectum were enlarged and caseous. The surface of the omentum was rough from the presence of a thin layer of partly organized fibrin. The omentum was thickened in places, but there were no distinct nodules. From the fact that the fibrinous coating of the serous membranes was as pronounced in the thoracic as in the abdominal cavity, it is probable that the culture of virulent tubercle bacilli injected intraperitoneally had little to do with the production of this deposit. This fibrinous coating may readily have resulted from the discharge of a pulmonary abscess into the pleural cavity or the discharge into the peritoneal cavity of the purulent contents of one of the softened lymphatic glands in the pelvis.

It is evident that the tubercle bacilli of human origin used for the inoculating of these two animals (26415 and 26442) even in the exceedingly large quantities in which they were employed, were incapable of causing general tuberculous infection. Even the intraperitoneal inoculation of the bull with a quantity of virulent culture nearly twice as great as was necessary when similarly administered to kill an unprotected heifer, did not so long as he was permitted to live, appreciably disturb his general health.

This experiment led us to believe that cattle were refractory to enormous quantities of tubercle bacilli from certain human sources, and further, that such injections were capable of increasing the normal resistance of cattle to virulent tubercle bacilli of bovine origin.

Another experiment was conducted in which four yearling calves were used. They were determined to be free from tuberculosis by the tuberculin test and were divided as near equally as possible in regard to size, condition, color, sex, etc., into two groups of two animals each. One group was immunized and the other group was kept under similar conditions as controls. The group selected to be vaccinated, Animals 45066 and 45068, were given seven intravenous injections of a standard suspension of Culture M of tubercle bacilli of human origin between March 24th and June 2nd, 1902, as follows:

March 24th, injected in right jugular 10 cc. Standard Suspension Culture M.

March 29th, injected in left jugular 15 cc. Standard Suspension Culture M.

April 3rd, injected in right jugular 20 cc. Standard Suspension Culture M.

April 9th, injected in left jugular 15 cc. Standard Suspension Culture M.

April 23d, injected in right jugular 20 cc. Standard Suspension Culture M.

May 20th, injected in left jugular 20 cc. Standard Suspension Culture M.

June 2nd, injected in right jugular 25 cc. Standard Suspension Culture M.

They received in all 125 cc. of the Standard Suspension.

On July 29th, or 57 days following the last vaccination, the two vaccinated animals as well as the two control animals were injected intratracheally with 10 cc. of a Standard Suspension of Culture H *bovine* tubercle bacilli. This culture was known to be highly virulent for cattle, as heretofore shown. The vaccinated animals and controls were killed with the following results:

Vaccinated animal, No. 45068, killed October 4th. A very careful autopsy revealed no evidence of tuberculosis in any part of the carcass excepting a slight fibrous thickening of the wall of the jugular veins at point of vaccination.

The second vaccinated animal was killed October 16th, and was found to be entirely normal excepting for two hard fibrous thickenings about 1-4 to 3-5 of an inch in diameter at the point of intratracheal infection on July 29th. These thickenings contained no caseous material, but were fibrous throughout.

A control animal, No. 45071, killed October 8th, showed an abscess 3-4 of an inch in diameter beneath the skin at the point of intratracheal injection. The mucous membrane of the trachea exhibited reddish, tuberculous elevations. The lungs contained caseous tuberculous nodules 1-4 to 3-4 of an inch in diameter evenly distributed throughout. One caseous area 2 inches in diameter found in apex of right lung. Nearly all the lymphatic glands associated with the pharynx and thoracic cavity were enlarged and caseous.

PLATE I.—Laboratory and post-mortem room at Experimental farm.

The second control animal, No. 45067, was killed October 16th. At the point of intratracheal injection the mucous membrane of the trachea contained many tuberculous nodules. The lungs and their associated lymphatic glands were evenly distributed with small foci of tuberculosis. The cervical and some of the mesenteric lymphatic glands were enlarged and caseous.

In brief, the two vaccinated animals contained no distinct lesion of tuberculosis, while the two controls contained widely distributed active progressive lesions. It might be well to note that these animals were kept during the experiment in a newly constructed barn and away from all infection to tuberculosis from natural sources. More detailed results of this experiment were published by the late Dr. Leonard Pearson and the writer in the Journal of Comparative Medicine and Veterinary Archives of November 1902, at which time we concluded:

1. "That after repeated intravenous injections of cultures of tubercle bacilli from human sputum, the resistance of young cattle to virulent tubercle bacilli of bovine origin may be increased to such an extent that they are not injured by inoculation with quantities of such cultures as are capable of causing death or extensive infection of cattle not similarly protected.

2. "That by intravenous injection much larger quantities of culture of human sputum tubercle bacilli than are necessary to confer a high degree of resistance or immunity upon the vaccinated animal may be administered without danger to that animal."

After obtaining these rather encouraging results a systematic plan was outlined and the Legislature was asked to supply the State Livestock Sanitary Board of Pennsylvania with funds for carrying on more extensive experiments to determine whether a practical method could be devised by which cattle might be immunized against tuberculosis. A number of the earlier experiments were carried out in connection with the Veterinary Hospital of the University of Pennsylvania. Later a pasture was rented near Philadelphia. In 1904 a farm of approximately 100 acres was purchased by the State in Delaware County for the conducting of experimental work upon animals, where most of the later experiments hereinafter recorded were carried on.

The culture of tubercle bacilli of human origin selected to produce immunity was the same culture as was used for the inoculation of heifer 26415 and bull 26442. It is known throughout all the experiments as culture M and was obtained from the sputum of a young adult that had a constant cough, though no distinct involvement of the lungs could be detected on physical examination. Smears made for microscopic examinations from the specimen of sputum showed an extraordinarily large number of tubercle bacilli. The organism was obtained in pure culture on dog's blood serum by the passage through guinea pigs on October 15, 1899. Miscroscopic examination of the bacilli revealed them to be irregular in size and shape. Many were curved and some S shaped. Beading was not marked. It stained rather deeply and fairly evenly. This culture at the time it was isolated and for some years afterwards, was found to be virulent for guinea pigs in ordinary doses, but not virulent for rabbits, goats or calves.

PREPARATION OF VACCINE.

The method of preparing the Vaccine or suspension of tubercle bacilli used throughout all the experiments was practically the same except where otherwise noted. A quantity of tubercle bacilli from a three to four week old glycerin bouillon culture was taken. The excess of moisture was removed by either blotting between layers or sterile filter paper or drying in a desiccator over calcium chloride for a short time. The bacilli were then triturated in a mortar or ground in a flask with bronze balls. During the process of triturating or grinding small quantities of normal saline solution were added from time to time. It was found that if a large quantity of saline solution were added at one time the bacilli had a tendency to clump and a homogeneous suspension could not be obtained. No effort was made to get the bacilli perfectly dry before grinding owing to the danger to the operator while triturating or grinding them in the flask. It was not the object to break up the bodies of the bacilli, but to separate them so that a homogeneous suspension could be obtained. The suspensions were allowed to stand in a cylindrical receptacle until all clumps of bacilli had settled to the bottom and then the supernatant homogeneous suspension of tubercle bacilli was decanted off. This suspension was further diluted with normal saline solution until its opacity was equal to a 24 hour old bouillon culture of typhoid bacilli. This method of measuring the dose suggested itself from the work of other investigators. Of course it was necessary to use precautions in preparing the Vaccine to prevent it from becoming contaminated. All lots of vaccine were cultured before injecting into animals to prove that they contained no extraneous organisms. In order to compare the dosage of Vaccine used in our experiments with the work of other investigators along a similar line, the amount by weight of dried tubercle bacilli per cc. of standard suspension was determined. This was done by taking a large quantity of killed tubercle bacilli and placing them in a desiccating chamber over calcium chloride until they were dry. These dry tubercle bacilli were then rubbed in a mortar and suspended in normal salt solution until the opacity was the same as the standard suspension of the Vaccine. By this method it was determined that

1 cc. of our standard suspension of Vaccine contained the equivalent of 0.0013 grams (1 3-10 milligrams) of dried tubercle bacilli. This equivalent was determined with a very large number of dried tubercle bacilli and therefore if any fraction of error existed it would be infinitesimal for 1 cc. of suspension. In some of the latter experiments the Vaccine was prepared by weight. First, the difference in weight was determined between a quantity of tubercle bacilli removed from a flask of bouillon and blotted between sterile filter paper and the same quantity after all moisture had been removed by drying in a vacuum over calcium chloride. This difference was determined on rather large quantity and the factor of loss in weight was computed for a gram of moist tubercle bacilli. The Vaccine was then prepared by weighing the moist tubercle bacilli and then by the use of the factor previously determined, the suspensions were prepared so that 1 cc. of the Vaccine represented the equivalent of 0.001 grams (1 milligram) of dried tubercle bacilli. This was believed to be more accurate than the opacity method. These suspensions of tubercle bacilli are termed throughout the experiments as our "Standard Suspension."

The Vaccine was generally used within a few hours after it was prepared. It was found that some lots of Vaccine had a tendency to clump if allowed to stand for any length of time. If the Vaccine was not used within a few hours following its preparation, it was kept in an ice-chest and cultural examinations made to determine its sterility. It was the object in all of the experiments to use Vaccine that was not over 48 to 72 hours old.

METHOD OF VACCINATION.

In all cases excepting where otherwise noted the Vaccine was injected into the jugular vein. The animal, depending upon its size, was either confined in a stanchion or cast. All of the larger animals could be successfully vaccinated without confining them other than in a stanchion. It was necessary to cast the small calves. (They would invariably jerk their head around and dislodge the needle after it had been inserted into the jugular vein). All methods of confining such as bull rings, etc., we found to be unnecessary, as they only had a tendency to excite the animal. The hair over the jugular vein was clipped with a pair of curved scissors and afterwards the skin was washed with a solution of carbolic acid or creolin. The jugular vein was filled by pressing the thumb at the lower portion of the neck. The needle was then inserted into the vein and a few drops of blood were allowed to pass through the needle to insure the same being open, as well as to be certain that the vein had been punctured. The syringe was then attached to the needle and the required dose injected directly into the blood stream. A syringe that can be readily sterilized and with accurate graduations is best adapted for this work. Of course it is best to have a slip needle, for the animal may struggle while the operator is attempting to attach a screw needle to the syringe, and therefore dislodge the needle from the jugular vein. The vaccinations were given alternately on the right and left side.

All of our experiments were outlined with a definite object in view and the plan of the experiment was carried out as closely as conditions would permit. In some instances, however, certain circumstances arose which made it necessary to change the original plan of the experiment to a certain extent.

EXPERIMENT 115 A.

The object of this experiment was to determine the immunizing value of intravenous injections of cultures of tubercle bacilli known to be non-virulent or of low virulence for cattle, and further, to determine the dosage and method best adapted to achieve this purpose. There were fourteen yearling animals in this experiment, nine of which were vaccinated by various methods and five were kept as controls. All of the animals prior to the starting of the experiment were found to be free from tuberculosis by the application of the tuberculin test. The animals to be immunized were divided into pairs or lots and each pair or lot will be described separately.

Animals No. 45183, a brindle steer, and No. 45184, a black heifer, were given ten intravenous vaccinations between November 27, 1902, and March 2, 1903. The amount of Vaccine administered at each vaccination ranged from 10 cc. to 30 cc. of a standard suspension of Culture M, the interval between vaccinations varying from eight to fourteen days. The exact date and dosage of the administration of the Vaccine is outlined as follows:

1902.

November 27th, injected in jugular vein, 10 cc. Standard Suspension Culture M.

December 4th, injected in jugular vein, 15 cc. Standard Suspension Culture M.

December 11th, injected in jugular vein, 20 cc. Standard Suspension Culture M.

December 23rd, injected in jugular vein, 25 cc. Standard Suspension Culture M.

1903.

January 10th, injected in jugular vein, 30 cc. Standard Suspension Culture M.

January 24th, injected in jugular vein, 15 cc. Standard Suspension Culture M.

January 31st, injected in jugular vein, 15 cc. Standard Suspension Culture M.

February 9th, injected in jugular vein, 15 cc. Standard Suspension Culture M.

February 18th, injected in jugular vein, 20 cc. Standard Suspension Culture M.

March 2d, injected in jugular vein, 20 cc. Standard Suspension Culture M.

Following the vaccination of the black heifer, No. 45184, on January 10th, the animal was noticed to be much depressed. The respirations were greatly increased, labored and painful. These animals were kept during the period of vaccination in a temporary stable in connection with the Veterinary Hospital of the University of Pennsylvania and were not exposed to any tuberculosis infection. On June 16th, 3½ months after the last vaccination, they were placed with their controls in a pasture with a number of tubercular cows. They were removed from this pasture five months later and stabled on the experimental farm with cows afflicted with advanced tuberculosis, and were kept with these animals until killed. These two animals were tuberculin tested on April 26, 1904, and the brindle steer, No. 45183, gave a maximum post-injection temperature of 103.4° F, while the black heifer, No. 45184, gave a maximum post-injection temperature of 103.8° F.

AUTOPSIES.

The brindle steer, No. 45183, was killed September 7, 1904, 1½ years from the time the last Vaccine was administered. Weight 779 lbs. Condition fair. On examination this animal showed a rather progressive tuberculosis of the pleurae, lungs, mediastinal and bronchial lymphatic glands, omentum, peritoneum, spleen and post-pharyngeal and mesenteric lymphatic glands. A striking feature in this case was the uniform distribution of the tuberculous areas in the lungs. The implantations had the appearance of being distributed there by the circulation.

The black heifer, No. 45184, which received the same number of vaccinations and treatment as the brindle steer, was killed at the same time. Weight 852 lbs. A very careful examination of all the organs, glands and membranes failed to detect any evidence of tuberculosis excepting in the posterior mediastinal lymphatic gland, which contained three caseous tubercles the size of a pea, all of which were well encapsulated with fibrous tissue.

It is the belief of the writer that the brindle steer, No. 45183, had a low natural resistance and possibly became infected from the too frequent and too large doses of Vaccine. This belief is based upon the character and distribution of the lesions in the lungs and other organs.

In the case of the black heifer, No. 45184, it was found that upon referring to one of the tuberculin tests prior to the experiment that this animal gave upon a re-test an after-temperature of 103.2° F., though she was considered at the time to be free from tuberculosis. The autopsy findings in this case suggest that the animal either had a small lesion of tuberculosis before entering the experiment or the immunity conferred by the Vaccine was lost before she was killed. Inoculations of guinea pigs from the lesion found in the mediastinal gland indicated that the bacilli had a much greater degree of virulence than the culture used for the Vaccine.

Two other animals of this experiment, a spotted bull, No. 45185, and a brown and white heifer, No. 16025, were vaccinated three times with a standard suspension of tubercle bacilli, twice with Culture M and once with Culture U, as follows:

BULL NO. 45185.

1902.

November 27th, injected in jugular vein, 10 cc. Standard Suspension of Culture M.

December 23rd, injected in jugular vein, 20 cc. Standard Suspension of Culture M.

1903.

January 24th, injected in jugular vein, 5 cc. Standard Suspension of Culture U.

HEIFER NO. 16025.

1903.

January 31st, injected in jugular vein, 10 cc. Standard Suspension of Culture M.

March 21st, injected in jugular vein, 20 cc. Standard Suspension of Culture M.

April 10th, injected in jugular vein, 5 cc. Standard Suspension of Culture U.

The Vaccine for the last vaccination of these animals was prepared from Culture U. This culture was isolated by Dr. M. P. Ravenel from the mesenteric gland of a child in December 1901. It was found to be highly virulent for guinea pigs, and a six weeks old calf inoculated intravenously with it on March 4, 1902, was killed in a moribund condition six weeks later, and showed extensive tuberculosis of the lungs, some of the nodules having gone on to the caseation stage. The Lymph Glands in the thoracic cavity were enlarged and miscroscopic examination of scrapings from the same revealed enormous numbers of tubercle bacilli. This culture was used for the third vaccination with the idea that the first two vaccinations with Culture M would give sufficient immunity to protect against any infection from Culture U, and therefore a higher resulting immunity would thereby be obtained. These two animals were exposed to natural infection by association with tubercular cows in the same manner as the controls. The exposure to the infection started on June 16, 1903.

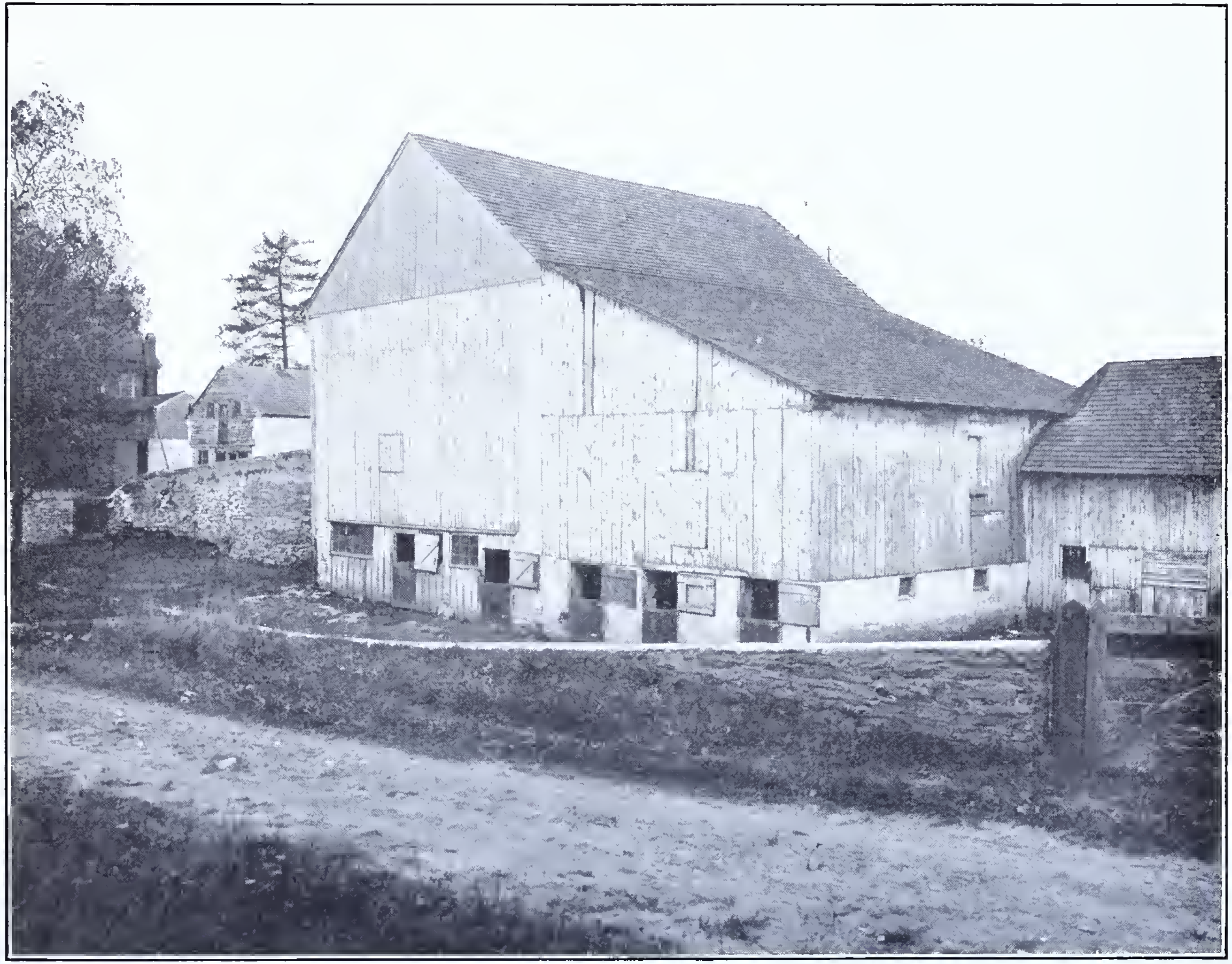

PLATE II.—Old barn in which immunized cattle were exposed to natural infection by association with tuberculous cows.

AUTOPSIES.

The spotted bull, No. 45185, died September 4, 1904, one year and 8½ months following the last vaccination. Weight 667 lbs. For three weeks this animal had been in poor condition and the cause of death was due to Tympanites. On postmortem the mediastinal lymphatic glands were enlarged and contained caseous material surrounded by a thick fibrous wall. The posterior mediastinal gland was 4″ x 6″ which forced the Oesophagus downward and may explain the Tympanites.

The brown and white heifer No. 16025 was killed September 7, 1904, one year and five months following the last vaccination. Weight 529 lbs. Condition fair. Had given birth to a calf two weeks prior to slaughter. There were three caseo-calcareous nodules found in the mediastinal lymphatic glands. Also three caseous areas 1-8″ to 1-3″ found in the mesenteric glands. These latter areas were sharply circumscribed and readily detached from the gland without disturbing the normal surrounding tissue. The condition was peculiar in that the yellow and comparatively caseous areas were firm in consistency and of a semi-cartilagenous feel. The postpharyngeal glands were somewhat enlarged and on section contained soft caseous material. There were two questionable lesions found in the left lung. A guinea pig inoculated with one of these nodules failed to develop tuberculosis.

It will be noted that the lesions in both of these animals were well circumscribed and walled off by fibrous tissue. From this and later knowledge obtained it seems more probable to the writer that the vaccination with Culture U following so closely upon the previous vaccination with Culture M, was the cause of the infection rather than from the association with the tubercular cows.

The fifth vaccinated animal of this experiment received on November 27, 1902, a single vaccination of 10 cc. of a standard suspension of Culture M. On January 20, 1903 this animal died of intestinal trouble and must be excluded from the experiment.

Another pair of vaccinated animals, a red bull, No. 45191, and a red steer, No. 45192, received between November 27, 1902, and January 24th, 1903, three intravenous vaccinations of a standard suspension of Culture M and two intravenous vaccinations of a standard suspension of Culture U. These two animals were approximately six to eight months of age at the start of the experiment and weighed 298 lbs. and 348 lbs. respectively. The Vaccine for each animal was administered as follows:

1902.

November 24th, injected in jugular vein, 10 cc. Standard Suspension Culture M.

December 4th, injected in jugular vein, 15 cc. Standard Suspension Culture M.

December 15th, injected in jugular vein, 20 cc. Standard Suspension Culture M.

1903.

January 10th, injected in jugular vein, 5 cc. Standard Suspension Culture U.

January 24th, injected in jugular vein, 5 cc. Standard Suspension Culture U.

On June 16, 1903, these animals were turned to pasture with tuberculous cows and later in the year were stabled at the experimental farm with animals badly afflicted with tuberculosis.

AUTOPSIES.

The red bull, No. 45191, was killed April 30, 1904, one year and three months following the last vaccination. Weight 680 lbs. Condition good. A careful autopsy failed to reveal any lesions of tuberculosis. All organs, glands, etc., normal.

The red steer, No. 45192, was killed April 26, 1904, one year and three months following the last vaccination. Weight not recorded. Condition fair. Both front knees of this animal had been greatly enlarged for three months and extremely painful. The capsules of knees were enlarged and filled with a pink gelatinous material. Between the two rows of carpal bones the cartilage was eroded. The lungs were found to contain a moderate number of tuberculous deposits about 1-8″ to 1-4″ in diameter. The bronchial and mediastinal lymphatic glands were enlarged and contained dense calcareous deposits. The viscera, the pleurae, diaphragm and peritoneum contained a few reddish growths.Microscopic examination of these deposits and growths showed no tubercle bacilli. However, animals inoculated from the lesions developed tuberculosis. A most striking feature was the sharpness with which the diseased areas were marked off from the normal tissue.

The eighth vaccinated animal, a red bull calf, No. 1606, received five intravenous vaccinations of a standard suspension of Culture M between May 16th and August 5th, 1903, the dose gradually increasing from 1 cc. on the first injection to 10 cc. on the last injection, as follows:

1903.

May 16th, injected in jugular vein, 1 cc. Standard Suspension Culture M.

May 23rd, injected in jugular vein, 3 cc. Standard Suspension Culture M.

June 12th, injected in jugular vein, 5 cc. Standard Suspension Culture M.

June 18th, injected in jugular vein, 7½ cc. Standard Suspension Culture M.

August 5th, injected in jugular vein, 10 cc. Standard Suspension Culture M.

On July 9, 1903, this animal was sent to pasture with the other animals of this experiment and was later stabled with tuberculous cows.

AUTOPSY.

It was killed on September 7, 1904, after fourteen months of exposure to natural infection. Weight 555 lbs. Condition fair. A careful examination revealed no evidence of tuberculosis excepting a calcified nodule 1-3 of an inch in diameter in the posterior lobe of the left lung. Guinea pigs inoculated from this lesion developed tuberculosis in six weeks.

The ninth and last vaccinated animal of this experiment, a red steer, No. 20027, which received five intravenous vaccinations of a standard suspension of Culture U between December 1, 1903, and July 14, 1904, as follows:

1903.

December 1st, injected in jugular vein, 10 cc. Standard Suspension Culture U.

1904.

March 17th, injected in jugular vein, 10 cc. Standard Suspension Culture U.

April 25th, injected in jugular vein, 10 cc. Standard Suspension Culture U.

June 30th, injected in jugular vein, 15 cc. Standard Suspension Culture U.

July 14th, injected in jugular vein, 10 cc. Standard Suspension Culture U.

On July 5th previous to the last vaccination this animal was removed to the experimental farm and exposed to natural infection by association with tuberculous cows. It was noticed that following each vaccination and for a few days thereafter the animal had a high evening temperature. This was especially noticed on the first two vaccinations. This animal was tuberculin-tested on September 5, 1904, two months following the last vaccination and gave no reaction.

AUTOPSY.

It was killed on December 30, 1905, one year and a half after exposure to infection. It was in good condition and a very careful examination of all the organs, glands and membranes failed to show any evidence of tuberculosis excepting in the left post-pharyngeal gland, which was 2½″ long x 1½″ thick. On section it was found to contain a cheesy mass 1″ in diameter. The lesion did not have the appearance of an active one.

The five control animals for this experiment, red steer, No. 45186, black bull, No. 45188, black and brown heifer, No. 45168, red bull, No. 45189, and red heifer, No. 45190, were kept under the same conditions as the vaccinated animals and exposed with them to the same degree of natural infection. These five controls were tuberculin-tested approximately one year following their exposure and all of them gave a typical tuberculin reaction.

AUTOPSIES OF CONTROLS AS FOLLOWS:

Red steer, No. 45186, killed April 30, 1904. Weight 813 lbs. Condition fair. Tuberculosis[1] of lungs TT. Pleuras TT. Post-pharyngeal glands, TT. Bronchial glands, TT. Mediastinal glands TTT. Peritoneum TTT. All the lesions appear to be active and progressive.

Black steer, No. 45188, killed September 7, 1904. Weight 814 lbs. Condition good. Active tuberculosis found in left lung, TT. Bronchial glands, TTT. Mediastinal glands, TTT.

Black and brown heifer, No. 45168. Killed September 7, 1904. Weight 624 lbs. Condition fair. Tuberculous lesions found in lungs TT. Bronchial glands, TTTT. Mediastinal glands, TTT.

Red bull, No. 45189. Killed April 30, 1904. Weight 638 lbs. Condition fair. Lesion of tuberculosis found in the lungs, TTTTT. Pleurae TT. Pericardium, TT. Cervical lymphatic glands, TT. Bronchial lymphatic glands, TTTT., Mediastinal lymphatic glands, TTTTT. Lymphatic glands anterior to heart and below trachea, TT. Liver, TT. Portal lymphatic glands, TT. Omentum, TT. Diaphragm, TT. In this animal the disease was rather widely distributed and of an active character.

Red heifer, No. 45190. Killed September 7, 1904. Weight 595 lbs. Condition fair. Lesions of tuberculosis found in lungs, TTT. Bronchial Lymphatic Glands, TTT. Mediastinal Lymphatic Glands, TTTT. Liver, TTTT. Diaphragm, T, Mesenteric Lymphatic Glands, TT.

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[1] The extent of the disease of the various organs, glands, membranes, etc. is indicated by the letter T. One T indicates the organ to be slightly involved, while six TTTTTT indicates extensive involvement of the organ, gland or tissue named.

EXPERIMENT 115D.

This experiment was started with the object of determining the size of the dose, the number of doses and the best interval between the injections of the Vaccine to produce in calves a serviceable degree of immunity against Tuberculosis by natural infection. Thirty calves ranging in age from ten to twelve weeks were selected for this purpose, twenty calves to be vaccinated and ten to be used as controls. These animals were purchased in Somerset County and were received in Philadelphia during a warm period of weather in July, 1903. They were given a tuberculin test at that time, which was only partially satisfactory owing to the age and condition of the animals. However, they were selected from apparently healthy herds. Following the tuberculin test they were removed from Philadelphia to a pasture in Montgomery County where they contracted lung worms (Strongylus Micrurus) during the period of vaccination and twelve of the thirty head died, leaving twelve upon which the vaccinations had been started and six controls. The animals to be vaccinated were divided into four lots and vaccinated as follows:

LOT I.

Contained three animals. A roan heifer, No. 16081, weight 275 lbs., a red and white heifer, No. 16087, weight 235 lbs. and a fawn heifer, No. 20002, weight 168 lbs. All of them were given four intravenous injections of a standard suspension of Culture M as follows:

1903.

August 9th, injected in jugular vein, 5 cc. of a Standard Suspension Culture M.

August 18th, injected in jugular vein, 10 cc. of a Standard Suspension Culture M.

December 6th, injected in jugular vein, 10 cc. of a Standard Suspension Culture M.

1904.

January 6th, injected in jugular vein, 15 cc. of a Standard Suspension Culture M.

LOT II.

Consisted of two animals. White heifer, No. 16089, weight 250 lbs. and brown and black bull, No. 16092, weight 159 lbs. These animals were given five intravenous vaccinations of a standard suspension of Culture M as follows:

ANIMAL NO. 16089.

1903.

August 5th, injected in jugular vein, 4 cc. Standard Suspension Culture M.

August 18th, injected in jugular vein, 8 cc. Standard Suspension Culture M.

1903.

September 10th, injected in jugular vein, 12 cc. Standard Suspension Culture M.

December 6th, injected in jugular vein, 12 cc. Standard Suspension Culture M.

1904.

January 6th, injected in jugular vein, 18 cc. Standard Suspension Culture M.

ANIMAL NO. 16092.

1903.

August 5th, injected in jugular vein, 4 cc. Standard Suspension Culture M.

August 18th, injected in jugular vein, 8 cc. Standard Suspension Culture M.

September 10th, injected in jugular vein, 10 cc. Standard Suspension Culture M.

December 6th, injected in jugular vein, 10 cc. Standard Suspension Culture M.

1904.

January 6th, injected in jugular vein, 18 cc. Standard Suspension Culture M.

It will be noted that the vaccination of these two animals was the same except on September 10th and December 6th, animal No. 16092 received only 10 cc. of Vaccine. At this time the animal was coughing from the lung worm infection and it was thought best not to give too large a dose of the Vaccine.

LOT III.

Consisted of three animals. Red steer, No. 16080, weight 328 lbs., brindle bull, No. 16093, weight 225 lbs., and red bull, No. 20005, weight 205 lbs. These animals were given in all five intravenous vaccinations of a standard suspension of Culture M. The dosage, however, was somewhat larger than for the preceding lot. The date and dosage is given as follows:

1903.

August 5th, injected in jugular vein, 5 cc. Standard Suspension Culture M.

August 18th, injected in jugular vein, 10 cc. Standard Suspension Culture M.

November 8th, injected in jugular vein, 15 cc. Standard Suspension Culture M.

December 6th, injected in jugular vein, 15 cc. Standard Suspension Culture M.

1904.

January 6th, injected in jugular vein, 20 cc. Standard Suspension Culture M.

Animal No. 16093 was not in good condition on December 6th and therefore did not receive its fourth vaccination until January 6th and its fifth vaccination until March 18th. The dosage, however, remained the same as for the other two animals.

LOT IV.

Consisted of four animals. Red bull, No. 16079, weight 355 lbs. red heifer, No. 16084, weight 260 lbs., brown and black heifer, No. 16095, weight 190 lbs. and a spotted bull, No. 16091, weight 268 lbs. These animals were given in all six vaccinations—five of a standard suspension of Culture M and one of a standard suspension of Culture U. The administration of the Vaccine for all the animals was as follows:

1903.

August 5th, injected in jugular vein, 5 cc. Standard Suspension Culture M.

August 18th, injected in jugular vein, 10 cc. Standard Suspension Culture M.

November 8th, injected in jugular vein, 15 cc. Standard Suspension Culture M.

December 6th, injected in jugular vein, 15 cc. Standard Suspension Culture M.

1904.

January 6th, injected in jugular vein, 20 cc. Standard Suspension Culture M.

March 18th, injected in jugular vein, 10 cc. Standard Suspension Culture U.

At the time of vaccination on August 18th, animal No. 16091 had a very high temperature and was given 8 cc. of Vaccine instead of 10 cc. the amount given the other three animals of this lot.

The six control animals for the four lots of vaccinated animals were as follows: A brown and white bull, No. 16082, weight 290 lbs., a red and white heifer, No. 20007, weight 164 lbs., a red and white heifer, No. 20008, weight 131 lbs., red heifer, No. 16086, weight 242 lbs., black bull, No. 16090, weight 260 lbs. and a red bull, No. 20003, weight 176 lbs.

All of the vaccinated animals including the controls were exposed during the period of vaccination to a moderate degree of infection by association in a pasture with tuberculous cows. Following the vaccinations they were removed to the experimental farm and all were constantly and evenly exposed to infection by being stabled with cows afflicted with advanced tuberculosis. The vaccinated

PLATE III.—Stable in which Vaccinated Cattle were kept with tuberculous animals.

animals and their controls were killed at various times to determine the progress of the experiment, and the findings are as follows:

RESULTS OF AUTOPSIES.

VACCINATED ANIMALS—LOT I.

Animal No. 16081. Killed December 30, 1904. Weight 580 lbs. All organs, glands, membranes, etc., normal.

Animal No. 16087. Killed April 8, 1905. Weight 622 lbs. All organs normal except there was found a pea sized nodule well encapsulated in the left post-pharyngeal gland. This nodule had all the appearances of an inactive lesion. It was calcareous throughout.

Animal No. 20002. Killed April 8, 1905. Weight 585 lbs. No evidence of tuberculosis could be found in any part of the carcass.

LOT II.

Animal No. 16089. Killed December 30, 1904. Weight 511 lbs. All organs, membranes, glands, etc., normal.

Animal No. 16092. Killed April 8, 1905. Weight 460 lbs. No evidence of tuberculosis in any part of the carcass.

LOT III.

Animal No. 16080. Killed September 15, 1904. Weight 406 lbs. No evidence of tuberculosis.

Animal No. 16093. Killed December 30, 1904. Weight 494 lbs. All organs, glands, membranes, etc., normal.

Animal No. 20005. Killed April 8, 1905. Weight, 535 lbs. Condition good. No evidence of tuberculosis found in any part of the carcass excepting in the left post-pharyngeal lymphatic gland there was a small nodule 1-8″ in diameter surrounded by a gray capsule. Guinea pigs inoculated from this nodule developed tuberculosis.

LOT IV.

Animal No. 16079. Killed September 15, 1904. Weight 480 lbs. No evidence of tuberculosis found. A small section in one of the lungs showed a chronic bronchial pneumonia, possibly the result of a previous infection by the Strongylus Micrurus.

Animal No. 16084. Killed December 30, 1904. Weight 431 lbs. No tuberculosis found in any part of the carcass. A small greenish nodule was found embedded in one of the lungs. Upon histological examination this nodule resembled a dense collection of calcified tissue rather than a tuberculous process.

Animal No. 16095. Killed April 8, 1905. Weight 580 lbs. No evidence of tuberculosis found.

Animal No. 16091. Killed April 8, 1905. Weight 710 lbs. All organs, membranes, etc., normal.

CONTROLS.

Animal No. 16082. Killed September 17, 1904. Weight 495 lbs. Early active lesions of tuberculosis were found in the lungs, TT. Post-pharyngeal glands, TT. Mesenteric glands, TTT.

Animal No. 20007. Killed December 30, 1904. Weight 410 lbs. Active tuberculosis found in the lungs, TTT. Bronchial Lymphatic Glands, TTTT. Mediastinal Lymphatic Glands, TTT. Liver, T.

Animal No. 20008. Killed December 30, 1904. Weight 416 lbs. The lungs contained one small area of suspected tuberculosis which was not proven by animal inoculation. Tuberculosis found in the Bronchial Lymphatic Glands, TT, and Mediastinal Lymphatic Glands, TTT.

Animal No. 16086. Killed April 8, 1905. Weight 634 lbs. Tuberculosis of the Post-pharyngeal Glands, TTT, and Omentum, TT. Lungs normal.

Animal No. 20003. Killed April 8, 1905. Weight 702 lbs. Active tuberculous processes found in the Post-pharyngeal Lymphatic Glands, TTT, and Mediastinal Lymphatic Glands, TTT. The border of the lungs showed a fringe of fibrin half an inch long, but contained no caseation.

Animal No. 16090. Died February 11, 1905. Weight 380 lbs. This animal had been in very poor condition for two months. A careful post-mortem examination revealed generalized tuberculosis of lungs, TTTTTT. Bronchial Lymphatic Glands, TTTT. Mediastinal Lymphatic Glands, TTTT. Pericardium, TT. Pleurae, TTTTTT. Diaphragm, TTT. Post-pharyngeal Lymphatic glands, TTT. Portal Lymph Gland, TT. Omentum, TTT.

The average length of exposure to infection for the vaccinated animals was 1 year, 172 days, while for the controls it was 1 year, 166 days. The average gain in weight of the vaccinated animals was 289 2-3 lbs. and the average gain in weight for the controls was 295 2-3 lbs. In briefly analyzing this exepriment we find that only two of the twelve vaccinated animals showed any evidence of tuberculosis on autopsy and in those the lesions were very small and inactive, while all of the controls presented lesions of tuberculosis distributed throughout the body. One of the controls, No. 16090, developed tuberculosis to such an extent that it died in one year and 190 days, showing that the degree of exposure to infection was rather severe.

EXPERIMENT 115E.

This experiment was started with the object of ascertaining the shortest and safest effective method of obtaining immunity in cattle against tuberculosis. The plan of the experiment, as written by the late Dr. Leonard Pearson, was as follows:

I. "Select seven yearling animals and carefully test them with tuberculin. Divide them as near equal as possible in regard to size condition, weight, etc., into lots of four and three animals respectively.

II. The lot of four animals are to be vaccinated as outlined in III and the lot of three animals are to be kept under similar conditions as controls.

III. METHOD OF VACCINATIONS.

The first animal of the lot to be vaccinated is to receive three vaccinations, the first injection to consist of 2 cc., the second 20 cc. and the third 40 cc. The second animal of this lot is to receive the same number of injections. The first injection, however, is to consist of 20 cc., the second 2 cc. and the third 40 cc. The third animal of the lot is to receive but one vaccination and to consist of the maximum safe dose as can be determined by the first two vaccinations of animals 1 and 2. The fourth and last animal of the lot is to receive two vaccinations consisting of the maximum safe dose as determined for animal 3 and repeated one time.

IV. All of the vaccinations are to be made with a standard suspension of Culture M. The intervals between the same are not to be less than 12 days, depending upon the temperature and condition of the animal.

V. Careful temperature measurements are to be taken and recorded morning and evening during the period of vaccination. The animals are to be weighed every seven to ten days. They are to receive like attention in regard to feed, care, etc.

VI. Method of Infection.

Three weeks after the date of the last vaccination all of the animals are to be infected by association with tubercular cows. In not less than eight months after the exposure to the infection the animals are to be killed and careful post-mortem notes recorded."

This plan was carried out as nearly as possible as outlined, as the following results of each animal will show. The animals were purchased in Center County and from herds that were known to be free from tuberculosis. They were tuberculin-tested on August 3, 1903, and passed a very satisfactory test.

VACCINATION OF ANIMALS.

Animal No. 20012. Red and white heifer. Weight 440 lbs.

1903.

September 11th, injected in jugular vein, 20 cc. Standard Suspension Culture M.

Animal No. 20013. Red heifer, weight 446 lbs.

1903.

August 6th, injected in jugular vein, 2 cc. Standard Suspension Culture M.

August 19th, injected in jugular vein, 20 cc. Standard Suspension Culture M.

October 10th, injected in jugular vein, 40 cc. Standard Suspension Culture M.

Animal No. 20015. Red and white heifer, weight 438 lbs.

1903.

August 6th, injected in jugular vein, 20 cc. Standard Suspension Culture M.

September 2d, injected in jugular vein, 2 cc. Standard Suspension Culture M.

October 10th, injected in jugular vein, 40 cc. Standard Suspension Culture M.

Animal No. 20016. Red heifer, weight 357 lbs.

1903.

September 11th, injected in jugular vein, 15 cc. Standard Suspension Culture M.

October 10th, injected in jugular vein, 25 cc. Standard Suspension Culture M.

Within ten minutes following the injection on October 10th of 25 cc. of the Vaccine, this animal showed great depression with pulse over 100, and rapid, labored and painful respirations. Animal died within 45 minutes. Autopsy revealed an acute extensive edema of the lungs. This animal was replaced by,

Animal No. 20018. Roan heifer, weight 372 lbs.
1903.

October 24th, injected in jugular vein, 15 cc. Standard Suspension Culture M.

December 7th, injected in jugular vein, 20 cc. Standard Suspension Culture M.

CONTROLS.

Animal No. 20114. Solid red heifer, weight 405 lbs.

Animal No. 20117. Small red heifer, weight 375 lbs.

Animal No. 20026, black and white bull, weight 180 lbs.

All of these animals were kept during the period of vaccination and until December 15, 1903, in a stable free from infection and after that date they were taken to the experimental farm in Delaware County where they were exposed to natural infection by association with tuberculous cows. It was desired to have the exposure as even as possible and a stable plan was outlined by which each animal was stanchioned adjacent to a diseased cow. The animals of the experiment were changed weekly from stanchion to stanchion in regular sequence, the tubercular cows remaining in the same stall.

We believed this method would give a more even exposure to infection.

RESULTS OF AUTOPSIES.

VACCINATED ANIMALS.

Animal No. 20012. Killed May 28, 1906—2½ years from date of exposure. Weight 730 lbs. Condition good. Tuberculous lesions found in the Posterior Mediastinal Gland, TT. Bronchial Glands, TT. Liver, TT. Portal Lymphatic Gland, T.

Animal No. 20013. Killed January 21, 1906, two years and one month of exposure. Weight 800 lbs. Condition good. Lesions of tuberculosis found in lungs, TT. Bronchial Glands, TT. Mediastinal Glands, TTT. Post-pharyngeal Glands, TT. Mesenteric Glands, TT.

Animal No. 20015. Killed May 28, 1906—2½ years of exposure. Weight 746 lbs. All organs, glands and membranes normal except one Mesenteric Lymphatic Gland, which contained a suspicious caseo-calcareous mass ½" in diameter. No animal inoculations were made to determine if this mass contained living tubercle bacilli.

Animal No. 20018. Killed May 28, 1906—2½ years of exposure. Weight 790 lbs. Condition good. The right lung contained a mass 1" x 2" which on section showed considerable fibrous tissue, surrounding collections of pus ¼" in diameter, which contained tubercle bacilli on microscopic examination. On histological examination a bronchial lymphatic node contained a tubercle with beginning caseation and tubercle bacilli. All other organs, glands and membranes normal.

CONTROLS.

Animal No. 20014. Killed September 17, 1904—nine months of exposure. Weight 610 lbs. Condition poor. Tuberculosis found in the left lung, TT. Right lung, TTT. Pericardium, TT. Left Bronchial Lymphatic Gland, TT. Mediastinal Lymphatic Gland, TTT. Mesenteric Lymphatic Glands, TT. The right inferior maxillary bone was enlarged and discharging pus through a fistulous tract, evidently Actinomycosis. There was apparently a double infection of the right lung,—Actinomycosis and Tuberculosis.

Animal No. 20017. Killed May 28, 1906—2½ years exposure. Weight 782 lbs. Condition fair. A tuberculous process 2" x 3" found in the posterior lobe of the left lung. The right lung contained lesions of Tuberculosis, TT. Mediastinal Lymphatic Glands, TTTT. Mesenteric Lymphatic Glands, TTT. Liver, T. Post-pharyngeal Lymphatic Glands, TT.

Animal No. 20026. Killed May 28, 1906—2½ years of exposure. Weight 840 lbs. Condition fair. Tuberculosis found in the lungs, TT. Bronchial Lymphatic Glands, TTTT. Mesenteric Lymphatic Glands, TTTT. Liver, TTT. Diaphragm, TT.

It will be noted that the animals of this experiment were exposed to infection for a longer period than called for in the plan. It will be seen that all of the vaccinated animals contained lesions upon autopsy. Animal No. 20012, which received only one vaccination, had no lesions in the lungs, but contained lesions in the associated Lymphatic Glands and Liver. Was this animal not protected by one vaccination, or was the immunity thus conferred so weak that it was lost before the 2½ years of exposure had expired. Animal No. 20013, which received a large initial dose of Vaccine followed by a small dose and then a large third dose, was not protected, while animal No. 20015, which received a small initial dose followed by two increasing doses, was protected to a greater degree than any of the others. However, none of the vaccinated animals showed as many or as wide a distribution of lesions as the controls. The average gain in weight of the vaccinated animals was 346¼ lbs., while the average gain in weight of the controls was 424 lbs. The condition of the vaccinated animals at time of slaughter was much better than the controls. The following tuberculin tests were made of these animals throughout the experiment:

TUBERCULIN TEST OF ANIMALS EXPERIMENT 115E. DECEMBER 16, 1904.

Number	December 13		December 14		December 15	December 16		Time of Injection	Dose	December 17					
	A. M.	P. M.	A. M.	P. M.	A. M.	A. M.	P. M.			5 A. M.	7 A. M.	9 A. M.	11 A. M.	1 P. M.	3 P. M.
20012,	102.6	101.8	102.4	101.2	102.2	102.	101.8	9 P. M.	.4 cc.	103.6	104.	106.	105.4	104.6	104.
20013,	102.2	101.4	101.4	101.6	101.6	101.6	101.8	"	"	104.	106.	106.8	106.8	106.2	105.8
20015,	101.4	102.	101.8	101.6	102.4	102.	100.8	"	"	102.8	105.4	106.2	106.	105.2	104.6
20017,	102.4	101.8	101.8	101.8	102.	102.2	102.2	"	"	102.2	105.4	106.	106.4	105.2	105.2
20018,	102.4	102.4	101.	102.	102.4	103.6	101.6	"	"	102.	103.2	104.2	105.4	104.2	104.2
20026,	101.8	102.2	101.	101.6	101.6	101.2	101.6	"	"	104.8	103.4	104.	103.8	104.	103.

TUBERCULIN TEST OF ANIMALS EXPERIMENT 115E. APRIL 5, 1905.

Number.	April 4th.		April 5th.				Time of Injection.	Dose.	April 6th.						April 7th.	
	10 A. M.	5 P. M.	7 A. M.	10 A. M.	2 P. M.	5 P. M.			6 A. M.	8 A. M.	10 A. M.	12 M.	2 P. M.	4 P. M.	8.30 A. M.	10.30 A. M.
20012,	99.	102.	102.2	101.	101.1	101.8	10 P. M.	.8 cc.	105.2	105.8	106.6	105.	100.	101.	100.8	101.
20013,	100.4	101.1	101.8	100.8	100.4	101.	"	"	105.	105.6	105.3	106.2	103.2	103.4	102.4	101.8
20015,	102.	99.2	101.2	101.8	101.	100.6	"	"	103.4	101.4	103.	103.6	104.6	100.4	102.	101.1
20017,	101.6	101.2	101.	99.2	101.	101.6	"	"	102.6	105.2	105.	104.6	101.1	98.6	102.4	101.
20018,	102.8	99.5	101.6	102.6	101.8	99.	"	"	102.2	102.6	102.4	102.	102.	99.5	101.7	102.
20026,	101.	101.2	102.6	102.	101.3	101.7	"	"	104.2	105.4	105.2	105.6	102.4	102.6	102.5	102.6

Watered between 12 M. and 2 P. M. April 6th.

TUBERCULIN TEST OF ANIMALS EXPERIMENT 115E. NOVEMBER 3, 1905.

Number.	Nov. 3, 1905.				Time of Injection.	Dose.	Nov. 4, 1905.							
	9 A. M.	11 A. M.	2 P. M.	4 P. M.			5 A. M.	7 A. M.	9 A. M.	11 A. M.	1 P. M.	3 P. M.	5 P. M.	7 P. M.
20012,	102.	101.7	102.	102.3	9 P. M.	1 cc.	102.	101.9	103.4	102.1	102.5	101.	102.2	....
20013,	100.5	101.	101.8	102.3	"	"	101.4	103.5	103.2	103.8	103.8	104.4	104.3	....
20015,	100.8	100.7	100.7	102.	"	"	100.8	101.3	101.6	101.5	101.5	101.5	101.6	....
20017,	102.	101.4	101.4	102.1	"	"	101.8	101.	102.	102.5	102.7	102.7	103.4	103.
20026,	101.2	100.5	101.7	102.2	"	"	101.5	104.	102.2	102.2	102.2	103.3	103.8	103.7
20018,	101.8	101.5	* 102.2	* 100.6	"	"	102.	101.3	102.4	† 101.4	† 101.	100.7	101.5	....

*November 16, 1905.
†November 17, 1905.

EXPERIMENT 115F.

This experiment was started with the object in mind of ascertaining whether a greater cell resistance against tuberculosis could be obtained by using a Vaccine in which the tubercle bacilli were suspended in the glycerine veal broth upon which they were grown and which contained the toxin liberated by them during their growth. In other words, to produce in an animal both a bacterial immunity and a toxic immunity. This experiment was not in any way satisfactory and very little knowledge was obtained upon the object in view when the experiment was outlined. The results are here recorded, as they have some value upon the preparation of the Vaccine.

The Vaccine was prepared as formerly described with the exception of the using of normal saline solution for the suspension of the tubercle bacilli. The glycerine veal broth upon which they had grown for six or more weeks was used as the medium. After the suspensions were prepared an effort was made to kill the tubercle bacilli by the addition of phenol or by heat. It will later be seen by the results that the addition of phenol in the instance of the bovine tubercle bacilli was not sufficiently strong or else was not allowed to remain in contact with the suspension long enough to kill the organisms.

Seven carefully tuberculin-tested animals were selected for the experiment. Five of them were to be vaccinated and the remaining two kept as controls. Three of the vaccinated group, a roan bull, No. 20020, weight 276 lbs., a red heifer, No. 20022, weight 290 lbs., and a black and white steer, No. 20024, weight 291 lbs., were injected intravenously seven times with a toxin broth suspension of standard opacity of tubercle bacilli of Culture M. The injections extended over a period from September 5, 1903, to June 30, 1904. The dosage varied for each animal and ranged from 5 cc. to 25 cc. at each injection. In some of the suspensions used for the injection the tubercle bacilli were killed by the addition of phenol, while in others they were killed by heat.

The remaining two animals of the vaccinated group, a Jersey heifer, No. 20021, weight 252 lbs., and a red and white heifer, No. 20023, weight 312 lbs., received on September 11, 1903, one intravenous injection of a toxin broth suspension of tubercle bacilli Culture H. This Culture was of bovine origin and possessed a high degree of virulence for cattle. After the suspension was prepared there was added 10% of a 5% solution of carbolic acid and the same was allowed to stand for 36 hours before being injected.

The other two animals in the experiment were kept as controls. A brindle steer, No. 20019, weight 352 lbs. and a red and white heifer, No. 20025, weight 252 lbs.

These animals during the period of vaccination and for two weeks after the same had been completed, were kept in a barn away from animals afflicted with tuberculosis. On July 5, 1904, all of the animals were taken to the experimental farm and exposed to natural infection by association with tuberculous cows.

They either died or were killed at various times with the following results:

VACCINATED ANIMALS.

Animal No. 20020. Killed September 10, 1904. This animal had been blind for some time. At the time it was removed from the place of vaccination to the experimental farm it was noticed that it would run against objects such as wagons and trees. The condition seemed to get better for a short time and then resulted in total blindness. On post-mortem examination it was found that the lungs were studded with gray miliary tubercles. The lung tissue was dense and non-elastic. No other lesions of tuberculosis found in any part of the body.

Animal No. 20022. Killed November 18, 1905,—exposure 1 year, 4½ months. No lesions of tuberculosis found in any part of the carcass except a small nodule in the right lung near the anterior border of the posterior lobe. Guinea pigs inoculated from the same developed generalized tuberculosis.

Animal No. 20024. Killed December 30, 1905,—exposure 1½ years. In one Mediastinal Lymphatic Gland and in one Bronchial Lymphatic Gland there were found a few caseous foci ⅛″ to ¼″ in diameter. All other organs, glands and membranes normal.

Animals No. 20021 and 20023, which received one injection of a toxin broth suspension of bovine tubercle bacilli, Culture H. Both died within four weeks following the injection. The autopsy revealed an acute miliary tuberculosis. Evidently the phenol had not killed the tubercle bacilli in the suspension in the time allowed, as these animals had not been exposed to any other infection.

CONTROLS.

Animal No. 20019. Killed November 18, 1905,—exposure 1 year, 4½ months. Extensive tuberculosis of the lungs, TTTTT. Mediastinal Lymphatic Glands, TTTTT. Post-pharyngeal Glands, TTTT. Mesenteric Lymphatic Glands, TTTT.

Animal No. 20025. Killed January 20, 1906,—exposure 1 year, 6½ months. The only lesion found in this animal was a small caseous tubercle in the anterior lobe of the right lung.

It does not seem possible to draw any conclusions of much value from these results further than the effect of phenol upon tubercle bacilli. It seems probable, however, that the toxin in the Vaccine may have been a factor in the causation of the blindness in animal No. 20020. Was the resistance of this animal lowered by the toxin and it became infected by the Vaccine, as the exposure to natural infection was only three months?

EXPERIMENT 115H.

This experiment was started with two objects in view:

I. To determine the quantity of Vaccine and the number of vaccinations required to produce a serviceable degree of immunity against tuberculosis in animals from natural infection.

II. To determine the length of time the immunity is retained by the animal.

The plan of the experiment as written by the late Dr. Leonard Pearson in January 1904 is as follows:

1. "Select thirty yearling heifers from a district which is practically free from tuberculosis.

2. Carefully test these animals with tuberculin and keep them all under similar conditions.

3. Divide them into two lots of twenty animals and ten animals each. The lot of twenty animals are to be vaccinated and the lot of ten animals to be kept as controls. The lot of twenty animals are again to be divided into five sub-lots of four animals each and to be known as Lots A, B, C, D, and E respectively. The animals in these lots are to be vaccinated according to the method described under paragraph 4.

4. METHOD OF VACCINATION.

Each member of Lot A is to receive two intravenous vaccinations of a standard suspension of tubercle bacilli, Culture M., the first vaccination to consist of 5 cc. and followed in twenty days with 25 cc.

Each member of Lot B is to receive two intravenous vaccinations of a standard suspension of Culture M, the first vaccination to consist of 5 cc. and followed in sixty days with 25 cc.

Each member of Lot C is to receive three intravenous vaccinations of a standard suspension of Culture M. The first vaccination to consist of 5 cc., the second in thirty days of 20 cc. and the third in thirty days from the second vaccination of 30 cc.

Each member of Lot D is to receive three intravenous vaccinations of a standard suspension of Culture M, the first vaccination to consist of 5 cc., and the second in sixty days of 30 cc., and a third in sixty days from the second vaccination of 40 cc.

Each member of Lot E is to receive no less than ten intravenous vaccinations of a standard suspension of Culture M. The first dose to consist of 10 cc. and the succeeding vaccinations to be increased gradually and to follow one another in as rapid succession as the condition of the animals will permit.

5. These animals are to be kept in the lower barn of the experimental farm until vaccinated. Each lot after the vaccinations have been completed for at least ten days, with two controls shall be moved up to barn No. 1 and exposed to natural infection by association with tuberculous cows.

6. The experiment will end with the slaughter and post-mortem examination of each animal used in the experiment."

This plan of the experiment was carried out as nearly as conditions would permit. The animals selected for this purpose were purchased in Center County and were all yearling heifers. Before the experiment was completed a number of them had given birth to calves, and the milk from those in Lot E was used for another experiment to determine whether the same had any immunizing

PLATE IV.—Lower Barn in which animals were vaccinated and stabled until exposed to infection.
See Experiment 115H.

value when fed to other animals. These animals all passed two satisfactory tuberculin tests before being entered into the experiment.

The animals of Lot A, Nos. 1, 5, 10 and 21 received two intravenous vaccinations at an interval of thirty days of a standard suspension of tubercle bacilli, Culture M, as follows:

1904.

January 6th, injected into jugular vein, 5 cc. Standard Suspension Culture M.

January 26th, injected into jugular vein, 25 cc. Standard Suspension Culture M.

On February 16, 1904, 21 days following the last vaccination, these animals were exposed to natural infection by being stabled with badly tuberculous cows.

The animals of Lot B, Nos. 4, 6, 8 and 27, received two intravenous vaccinations at an interval of sixty days of a standard suspension of tubercle bacilli, Culture M, as follows:

1904.

January 6th, injected into jugular vein, 5 cc. Standard Suspension Culture M.

March 26th, injected into jugular vein, 25 cc. Standard Suspension Culture. M.

On March 19, 1904, thirteen days following the last vaccination, the animals of this lot were exposed to natural infection the same as Lot A.

The animals of Lot C, Nos. 17, 18, 20 and 25, received three intravenous vaccinations of a standard suspension of tubercle bacilli, Culture M, as follows:

1904.

January 6th, injected in jugular vein, 5 cc. Standard Suspension Culture M.

February 16th, injected in jugular vein, 20 cc. Standard Suspension Culture M.

March 19th, injected in jugular vein, 30 cc. Standard Suspension Culture M.

These animals were exposed shortly after the last vaccination to natural infection by association with tuberculous cows.

The animals of Lot D, Nos. 13, 14, 23 and 26, received three vaccinations of a standard suspension of tubercle bacilli Culture M, as follows:

1904.

January 6th, injected in jugular vein, 5 cc. Standard Suspension Culture M.

March 6th, injected in jugular vein, 30 cc. Standard Suspension Culture M.

May 8th, injected in jugular vein, 40 cc. Standard Suspension Culture M.

The exposure of these animals to infection started before the vaccinations were completed, though the degree of exposure became more severe shortly following the last vaccination.

The animals of Lot E, Nos. 11, 15, 16 and 28 received ten intravenous vaccinations of a standard suspension of tubercle bacilli Culture M, as follows:

1904.

January 6th, injected in jugular vein, 10 cc. Standard Suspension Culture M.

January 26th, injected in jugular vein, 20 cc. Standard Suspension Culture M.

February 16th, injected in jugular vein, 20 cc. Standard Suspension Culture M.

March 6th, injected in jugular vein, 20 cc. Standard Suspension Culture M.

March 19th, injected in jugular vein, 15 cc. Standard Suspension Culture M.

April 2nd, injected in jugular vein, 20 cc. Standard Suspension Culture M.

April 15th, injected in jugular vein, 15 cc. Standard Suspension Culture M.

April 26th, injected in jugular vein, 10 cc. Standard Suspension Culture M.

May 2nd, injected in jugular vein, 10 cc. Standard Suspension Culture M.

May 8th, injected in jugular vein, 20 cc. Standard Suspension Culture M.

On March 19th these animals were exposed to a moderate degree of natural infection which became more severe after the vaccinations were completed. The ten control animals, Nos. 2, 3, 7, 9, 12, 19, 22, 24, 29 and 30 were kept under similar conditions as the twenty vaccinated animals. There were two controls for each lot of four vaccinated animals. The controls received the same degree of exposure as the animals that were vaccinated. All of the animals were constantly exposed until they died or were killed with the following results:

RESULTS OF AUTOPSIES.

VACCINATED ANIMALS—LOT A.

Animal No. 1. Killed December 6, 1904, 9¾ months of exposure. Weight 675 lbs. All organs, glands, membranes, etc., normal.

Animal No. 5. Killed December 30, 1905, 1 year, 10½ months of exposure. The middle Mediastinal Lymphatic Gland contained a caseous focus ¼″ in diameter. The left Post-pharyngeal Lymphatic Gland was enlarged and contained a small cavity ¾″ in diameter, which was filled with greenish pus. This pus, however, infected guinea pigs on inoculation. Six of the Mesenteric Lymphatic Glands were found to contain caseous foci varying in size from ½″ to ¾″ in diameter.

Animal No. 10. Killed January 2, 1907, two years 10½ months of exposure. One encapsulated nodule ¾″ in diameter in the left lung. A few Mesenteric Lymphatic Glands contained encapsulated caseous foci. The left Post-pharyngeal Gland contained one cheesy focus ½″ in diameter. All lesions were well encapsulated. All other organs normal.

Animal No. 21. Killed January 1, 1907, 2 years, 10½ months of exposure. Lesions of tuberculosis found in lungs, TT; Mediastinal Lymphatic Glands, TTT; Liver, TTT, and Portal Lymphatic Gland, TT.

LOT B.

Animal No. 4. Killed January 1, 1907, 2 years, 10½ months of exposure. Tuberculosis found in the lungs, TT; left Bronchial Lymphatic Gland, T; right Post-pharyngeal Lymphatic Gland, TT; Mesenteric Lymphatic Glands, T.

Animal No. 6. Killed December 30, 1905, one year, 10½ months of exposure. Lesions of tuberculosis found in the lungs, TTT; Bronchial Lymphatic Glands, TTT; Mediastinal Lymphatic Glands, TTT; right Post-pharyngeal Lymphatic Gland, T.

Animal No. 8. Killed January 9, 1907, 2 years, 10¾ months of exposure. Lungs normal. Lesions of tuberculosis found in left Bronchial Gland, T; Mesenteric Glands, TTT, and Livèr, T. All the lesions in this animal were distinctly encapsulated.

Animal No. 27. Killed January 9, 1907, 2 years, 10¾ months of exposure. One nodule ¾″ in diameter found in the left lung. One Mesenteric Lymphatic Gland contained three perfectly dry calcareous nodules. All other organs normal.

LOT C.

Animal No. 17. Killed January 21, 1906,—approximately two years of exposure. No lesions of tuberculosis found.

Animal No. 18. Killed December 30, 1905, approximately 23 months of exposure. Normal throughout except a small caseo-calcareous nodule ¼″ in diameter in the left Bronchial Lymphatic Gland. This nodule was well encapsulated and in a latent condition.

Animal No. 20. Killed January 20, 1907, approximately three years of exposure to infection. Lesions of tuberculosis found in lungs, T; Bronchial Lymphatic Glands, TT; Mediastinal Lymphatic Glands, T, and in one Mesenteric Gland, T. These lesions were very slight, but widely distributed.

Animal No. 25. Killed January 1, 1907,—approximately three years of exposure. Lesions of tuberculosis found in the lungs, TTT; Liver, TTT; Portal Lymphatic Gland, TTT; Mesenteric Lymphatic Glands, TT, and Omentum, T.

LOT D.

Animal No. 13. Killed January 2, 1907,—approximately two years, ten months of exposure. Lungs normal. Tuberculous lesions found in the Mediastinal Lymphatic Glands, TTT; Bronchial Lymphatic Glands, TT, Mesenteric Lymphatic Glands, TTT, and Post-pharyngeal Lymphatic Glands, TT. All of these lesions were distinctly fibrous.

Animal No. 14. Killed April 24, 1905,—approximately 1 year of exposure. No tuberculosis found in any part of the carcass. It was killed on account of a lung abscess due to a foreign body from rumen.

Animal No. 23. Killed January 21, 1906,—approximately 1 year, 10 months of exposure. No lesions of tuberculosis of any of the organs, glands or membranes.

Animal No. 26. Killed January 9, 1907, approximately 2 years, 10 months of exposure. Lungs normal except for some new formed fibrous tissue on pleural covering. Lesions of tuberculosis found in the left Bronchial Lymphatic Glands, TT; Mediastinal Lymphatic Glands, TT; right Post-pharyngeal Lymphatic Glands, TT, and one Mesenteric Lymphatic Gland, T.

LOT E.

Animal No. 11. Killed July 21, 1906,—approximately two years of exposure. Lesions of tuberculosis found in lungs, TT; left Bronchial Lymphatic Gland, T; Pericardium, T; Costal Pleura opposite Pericardium, T; Mediastinal Lymphatic Glands, TTT; Post-pharyngeal Lymphatic Glands, TT. There was an abscess back of the Pharynx and connecting with the same. Several of the Lymphatic Glands adjacent to this abscess were firm and caseous.

Animal No. 15. Killed January 9, 1907,—approximately 2 years, 9 months of exposure. All organs, glands, membranes, etc., apparently normal from macroscopic examination. A section from the Posterior Mediastinal Lymphatic Gland upon histological examination showed in the center of the section several giant cell-like bodies. No evidence of caseation or cellular infiltration. However, guinea pigs inoculated from an emulsion of this gland developed tuberculosis. Guinea pigs inoculated from other organs and glands of this animal remained healthy.

Animal No. 16. Killed September 19, 1904, approximately six months of exposure. No evidence of tuberculosis could be found in any part of the body.

Animal No. 28. Killed January 9, 1907, approximately 2 years and 10 months of exposure. No lesions of tuberculosis found except in one Post-pharyngeal Lymphatic Gland, which on section contained a thick fluid not entirely characteristic of tuberculosis. Guinea pigs inoculated from the same developed tuberculosis, while guinea pigs inoculated from the Liver, Spleen, Kidneys, Lung and Mediastinal Lymphatic Glands remained healthy.

CONTROL ANIMALS.

Animal No. 2. Killed November 4, 1904, eight months of exposure to infection. General condition bad. Extensive tuberculosis of Lungs, Bronchial and Mediastinal Lymphatic Glands. These tissues scored to the extent of TTTTTT or the highest degree in the scale.

Animal No. 3. Killed January 10, 1907, 2 years, 10¾ months of exposure to infection. Animal in poor condition. Tuberculosis of Lungs, TTT; Bronchial Lymphatic Glands, TTT; Liver, TT; Portal Lymphatic Gland, T; One Kidney, T; Mesenteric Lymphatic Glands, TT; Post-pharyngeal Lymphatic Glands, TTT.

Animal No. 7. Died May 7, 1904, two months following exposure. The autopsy notes of this animal were lost, but the physical condition of the animal prior to death was bad.

Animal No. 9. Killed January 10, 1907, two years, 10¾ months of exposure to infection. Lesions of tuberculosis found in the Lungs, TTTT; Bronchial Lymphatic Glands, TTTT; Mediastinal Lymphatic Glands, TTTT; Mesenteric Lymphatic Glands, TT; Post-pharyngeal Lymphatic Glands, TTT, and superior Cervical Lymphatic Glands, TT.

Animal No. 12. Died January 14, 1906, 1 year, 10 months of exposure to infection. This animal had been in poor condition for some time. Lesions of tuberculosis found in the Lungs, TTTTTT. Some of the caseous masses were 10″ in diameter. Lesions in the Bronchial Lymphatic Glands, TTT; right Post-pharyngeal Glands, TT; Portal Lymphatic Gland, TT, and Mediastinal Lymphatic Glands, TTTTT.

Animal No. 19. Killed January 10, 1907, approximately 2 years, 10 months of exposure. Lesions of tuberculosis found in the Lungs, TT; Bronchial Lymphatic Glands, TTT; Mediastinal Lymphatic Glands, TT; Portal Lymphatic Gland, T, and Mesenteric Lymphatic Glands, T.

Animal No. 22. Killed January 10, 1907, approximately 2 years, 10 months of exposure to infection. Lesions of tuberculosis found in the Lungs, TTTT; Bronchial Lymphatic Glands, TTT; Mediastinal Lymphatic Glands, TTTT, and Post-pharyngeal Lymphatic Glands, TT. Some of the diseased masses found in the lungs were as large as 4″ in diameter.

Animal No. 24. Killed January 10, 1907, approximately 2 years, 10 months of exposure to infection. Lesions of tuberculosis found in the Lungs, TTT; Pleura, TT; left Bronchial Lymphatic Gland, T, and Posterior Mediastinal Lymphatic Gland, T.

Animal No. 29. Died March 7, 1906, approximately 2 years of exposure to infection. This animal had been in poor condition for three months. Coughed badly. Refused grain and had to be assisted to regain feet when down. Extensive generalized tuberculosis of the organs, glands and membranes of both the thoracic and abdominal cavities. Also, the Post-pharyngeal Lymphatic Glands. This was one of the worst cases of tuberculosis among the experimental animals. The disease was more widely distributed and more extensive than in a number of the tuberculous cows that were used for infection purposes.

Animal No. 30. Killed January 10, 1907, approximately 2 years, 10 months of exposure to infection. Lesions of tuberculosis found in the Lungs, TT; Posterior Mediastinal Lymphatic Glands, T; Mesenteric Lymphatic Glands, T, and Omentum, T. The disease in this animal was not very extensive or widely distributed.

In making a brief analysis of the results of this experiment it is seen that the controls were more extensively diseased and the lesions in them were more widely distributed. Three of the controls died within two years from the time the exposure to infection began, showing that the degree of exposure was rather severe. All of the vaccinated animals lived until killed. Five of the vaccinated animals, Nos. 1, 17, 14, 23 and 16, showed no lesions of tuberculosis whatever. Animal No. 15 showed no microscopic lesions, but guinea pigs inoculated from a congested area in the post-pharyngeal Lymphatic Gland

PLATE V.—Barn and yard in which vaccinated calves were allowed to run with their tuberculous dams.

PLATE VI.—Vaccinated Calves and their Controls exposed to infection.

developed tuberculosis. Three of the remaining vaccinated animals, Nos. 27, 18 and 28 had very slight lesions, in some instances only a nodule $\frac{1}{4}$" in diameter. The lesions in the controls were active and progressive, while those found in the vaccinated animals had the appearance in many instances of being latent or retrogressive. It is possible that the immunity given the animals in Lots A and B had been lost before they were killed, as these animals had received only two vaccinations. From the character of the lesions and from other experience the writer believes that the animals in Lots C, D and E may have become infected during the period of vaccination, as they were partially exposed at that time. The lesions in these animals were not characteristic of a progressive tuberculosis found in the usual autopsies of animals slaughtered under the tuberculin test.

EXPERIMENT 115 I.

This experiment was started with the object of determining whether a tuberculosis free herd could be reared from animals a large percentage of which was afflicted with tuberculosis, many of them in the advanced form of the disease. Also, to obtain further knowledge upon the best culture of tubercle bacilli for the preparation of the Vaccine as well as the dosage and number of vaccinations required to produce a serviceable degree of immunity

The animals used in this experiment were the calves from the tuberculous cows that were kept for infection purposes, as well as the calves from the vaccinated and control animals in the foregoing experiments. The method employed was to vaccinate the calf within three to twelve weeks from birth or as soon as it was strong enough to withstand the initial dose of Vaccine. The number of doses of Vaccine given was from two to four and in most cases three doses. The initial dose was 1 cc. or $2\frac{1}{2}$ cc., depending upon the condition, size and age of the calf. Each subsequent dose was increased. No regular plan of increasing the dose was followed. The maximum dose at the last injection was never more than 10 cc.

During the early part of the experiment the calves were not removed from their dams in the infected stables and therefore were receiving milk that contained tubercle bacilli. The stables in which they were kept were good sources of infection. It was found during the progress of this experiment that the resistance of a calf against tuberculosis during the period of vaccination and for a few weeks thereafter, was less than the normal resistance of an animal, and if the calf was exposed to infection at the time of vaccination it was more liable to contract tuberculosis than the normal controls. The autopsy findings on such calves indicated that the infection implantations took place more readily, but the disease did not progress as rapidly as in the controls.

There were 22 calves vaccinated during the time they were allowed to remain in the infected stables with their tuberculous mothers. These calves were continually exposed to infection and either died or were killed within 2½ years following the last vaccination. At autopsy 20 of the 22 animals showed lesions of tuberculosis, as will be seen in the following table:

TABLE OF CALVES VACCINATED DURING PERIOD OF EXPOSURE TO INFECTION.

Animal No.	Dam	Date of birth	Vaccinations				Culture	Exposed to infection from tub. cows	Interval between last Vac. and exposure	Killed or died	Postmortem Findings
			1st.	2nd.	3rd.	4th.					
No number, ...	Tub. cow 20036	8-15-03	9-10-03 1 cc.	10-3-03 5 cc.	11-8 03 7½ cc.	12-6-03 7½ cc.	M.	From birth	None, ..	Died 2-7-04	No tuberculosis. Bronchial pneumonia due to lung worms.
58, ...	Tub. cow 20038	8-23-03	9-11-03 1 cc.	10-3-03 5 cc.	11-8-03 7½ cc.	12-6-03 7½ cc.	M.	From birth	None, ...	Died 8-24-06	Mesenteric glands TT. Very calcareous, hard, dry and encapsulated lesions.
59, ...	Tub. cow 20035	9-13-03	9-24-03 1½ cc.	11-8-03 7 cc.	12-6-03 5 cc.	......	M.	From birth	None. ...	Killed 9-17-04	Left bronchial. Medistinal. All lesions calcified.
No number, ...	Tub. cow 20037	10-8-03	11-8-03 1 cc.	12-6-03 3 cc.	......	......	M.	From birth	None, ...	Died 3-11-04	Tuberculosis of lungs TTT. Mediastinal Glands T. Strongylus Micrurus in Lungs.
62, ...	Tub. cow 34	4-15-04	4-26-04 1 cc.	5-25-04 2½ cc.	6-15-04 4 cc.	7-14-04 6 cc.	M.	From birth	None, ...	Killed 9-17-04	Spleen TTT. Liver, TT. Portal gland TT. Evidently prenatal infection. Dam had tub. ovaries.
77, ...	Animal 19	5-21-04	6-15-04 2½ cc.	7-14-04 4 cc.	8-11-04 5 cc.	......	M.	From birth	None, ...	Killed 12-30-05	Normal.
92, ...	Animal 23	6-27-04	7-14-04 1 cc.	8-11-04 3 cc.	......	......	M.	From birth	None. ...	Died 9-23-06	General tuberculosis.
159, ...	Tub. cow 110	12-4-04	12-11-04 ½ cc.	2-16-05 2½ cc.	......	......	M.	From birth	None, ...	Died 9-25-07	General tuberculosis.
173.	Tub. cow 116	12-27-04	12-30-04 ½ cc.	2-16-05 2½ cc.	......	......	M.	From birth	None, ...	Killed 9-27-07	Lungs TTTT, Bronchial Glands TTT, Mediastinal TTT.
279, ...	Tub. cow 36	2-19-05	3-16-05	5-13-05	8-7-05	......	M.	From birth	None, ...	Killed 1-2-07	Lungs TT, Bronchial glands TT, Mediastinal glands, TT, Liver TT, Spleen T, Mesenteric glands TT, Post-phar. TT.
338, ...	Animal 147	4-13-05	10-5-05 2 cc.	12-4-05 5 cc.	1-24-06 10 cc.	4-25-06 12½ cc.	M.	From birth	None. ...	Killed 1-1-08	Nodule ⅛″ in diam. in each lung. Nodule 1-16″ in diam. in both bronchial glds. Nodule in Mediastinal gld. All lesions very dry and encapsulated.

TABLE OF CALVES VACCINATED DURING PERIOD OF EXPOSURE TO INFECTION—Concluded.

Animal No.	Dam	Date of birth	Vaccinations				Culture	Exposed to infection from tub. cows	Interval between last Vac. and exposure	Killed or died	Postmortem Findings
			1st.	2nd.	3rd.	4th.					
409, ...	Tub. cow 111	1-3-06	2-11-06 1½ cc.	4-5-06 4 cc.	5-28-06 6 cc.	7-23-06 8 cc.	M.	From birth	None, ...	Killed 1-1-08	Post Mediastinal and left bronchial glands contain tub. foci. 1-6″ in diam. So dry and calcareous could hardly cut.
410, ...	Tub. cow 110	1-5-06	2-11-06 1½ cc.	4-5-06 4 cc.	5-28-06 6 cc.	......	M.	From birth	None, ...	Killed 10-11-06	Lungs TT, Mediastinal glds, T. Post-pharyngeal TT.
416, ...	Animal 30	12-26-05	2-11-06 1½ cc.	4-5-06 4 cc.	5-28-06 6 cc.	7-23-06 8 cc.	M.	Partial exposure fm. birth	None, ...	Killed 1-2-07	One nodule ½″ in right lung. One nodule ¾″ in Mediastinal Gland. All other organs normal.
418, ...	Animal 9	1-11-06	2-11-06 1½ cc.	4-5-06 4 cc.	5-28-06 6 cc.	......	M.	From birth	None, ...	Killed 7-31-06	A moderate degree of tuberculosis of all organs. Infection believed to be bovine by rabbit inoculation.
424, ...	Animal 24	2-2-06	2-11-06 1 cc.	4-5-06 3 cc.	5-28-06 6 cc.	7-23-06 8 cc.	M.	From birth	None, ...	Killed 1-1-08	Pleura thickened. No tubercles in any part of carcass.
425, ...	Animal 25	1-27-06	2-11-06 1 cc.	4-5-06 3 cc.	......	......	M.	From birth	None, ...	Killed 6-16-06	Bad condition. General tuberculosis of lungs and associated glands.
444, ...	Vac. Animal 180	3-4-06	4-28-06 1 cc.	6-8-06 3 cc.	......	......	M.	From birth	None, ...	Killed 1-2-07	Three caseous foci in one lung about ⅛″ in diameter. All other organs normal.
449, ...	Tub. cow 112	3-11-06	4-28-06 1 cc.	6-8-06 3 cc.	......	......	M.	Partial from birth	None, ...	Killed 11-6-08	One nodule ¾″ in lungs. Bronchial glds. Post-pharyngeal glds. Lesions latent.
451, ...	Tub. cow 116	3-16-06	4-28-06 3 cc.	6-8-06 3 cc.	7-20-06 6 cc.	......	M.	Partial from birth	None, ...	Killed 11-5-08	Only lesion is a small nodule in one Mesenteric Gland.
455, ...	**Vac. Animal 20118**	3-22-06	**4-28-06 2 cc.**	**6-8-06 2 cc.**	**7-20-06 10 cc.**	......	**M.**	From birth	None, ...	Died 12-31-06	No distinct lesions of tuberculosis. Death due to abscess following treatment for lung worms.

456, ...	Animal 20017	3-24-06	5-26-06 4 cc. Intravenously	8-29-06 20 cc. Intraperitoneally	......	......	M.	Partial from birth	None, ...	Killed 11-6-08	Normal.

In the latter half of the experiment the calves were removed from their tuberculous mothers and the infected stables as soon after birth as possible, and kept in a special stable away from all infection and fed on milk that had been pasteurized or boiled. There were nine calves treated in this manner and were not vaccinated until they were three to four months old, and then received three injections of Vaccine prepared from tubercle bacilli, Culture M. The dosage was increased at each vaccination. After the vaccinations had been completed for some time they were exposed to infection by association with tuberculous cows. These nine animals were killed approximately three years following the vaccination and all of them were normal in every respect excepting two, one of which showed a small lesion of tuberculosis in the Mesenteric Gland and the other a small nodule in the Mediastinal Gland. Is it not possible that the immunity in these two animals had been lost during their three years of exposure? The following table will show the number of vaccinations, the dosage, the origin of the calf, etc.

TABLE OF CALVES KEPT DURING PERIOD OF VACCINATION AWAY FROM ALL EXPOSURE TO INFECTION.

Animal No.	Dam	Date of birth	Vaccinations				Culture	Exposed to infection from tub. cows	Interval between last Vac. and exposure	Killed or died	Postmortem Findings
			1st.	2nd.	3rd.	4th.					
661, ...	Tub. cow 113	11-11-06	4-8-07 5 cc.	7-11-07 10 cc.	9-10-07 15 cc.	......	M.	1-5-08	4 mo.	Killed 8-4-10	Normal.
733, ...	Purchased from Carpenter	12-1-06	4-8-07 5 cc.	5-22-07 7½ cc.	7-13-07 10 cc.	......	M.	11-6-07	3¾ mo.	Killed 8-11-10	Normal.
739, ...	Vac. Animal 179	12-24-06	4-8-07 2½ cc.	5-22-07 5 cc.	7-13-07 7½ cc.	......	M.	1-5-08	5¾ mo.	Killed 8-5-10	Normal.
740, ...	Animal 145	1-4-07	4-8-07 4 cc.	5-22-07 6 cc.	7-13-07 8 cc.	......	M.	1-5-08	5¾ mo.	Killed 8-4-10	Five mesenteric glands slightly enlarged and contain soft cheesy material. A suspicious condition in udder. However, not proven to be tuberculosis. Other organs normal.
742, ...	Tub. cow 110	1-16-07	4-8-07 4 cc.	5-22-07 6 cc.	7-13-07 8 cc.	......	M.	11-26-08	4½ mo.	Killed 8-11-10	Normal.
746, ...	Animal 60	3-6-07	4-8-07 2 cc.	6-6-07 3 cc	7-24-07 5 cc.	......	M.	1-5-08	5½ mo.	Killed 8-11-10	A few firm gray yellowish, distinctly calcareous points in Mediastinal glands. All other organs normal.
747, ...	Tub. cow 112	3-9-07	4-8-07 2 cc.	7-11-07 4 cc.	9-10-07 6 cc.	......	M.	1-5-08	4 mo.	Killed 8-4-10	Normal.
748, ...	Tub. cow	3-11-07	4-8-07 1 cc.	6-6-07 2 cc.	7-24-07 4 cc.	......	M.	8-26-08	13 mo.	Killed 2-7-10	Normal.
750, ...	Tub. cow	3-20-07	5-22-07 1 cc.	7-13-07 2½ cc.	9-10-07 5 cc.	......	M.	10-30-08	13½ mo.	Killed 8-16-10	Normal.

There were seven calves vaccinated with tubercle bacilli prepared from cultures of various sources. These cultures were used to determine whether better results could be gotten than with Culture M used for the former experiments. The following table does not indicate that the human culture B. T. and R. R. and the Avian Culture P. I. have any greater immunizing power than Culture M.

PLATE VII.—Barn in which calves were kept after removal from their tuberculous mothers and until the vaccinations were completed. Experiment 115 I.

TABLE OF CALVES VACCINATED WITH CULTURES OTHER THAN M.

Animal No.	Dam	Date of birth	Vaccinations				Culture	Exposed to infection from tub. cows	Interval between last Vac. and exposure	Killed or died	Postmortem Findings
			1st.	2nd.	3rd.	4th.					
70, ...	Tub. cow 42	......	3-17-04 2 cc.	6-7-04 2 cc.	7-14-04 3 cc.	......	Avian Culture P. I. 1	From birth	None, ...	8-25-05	Lungs TT, Left Bronchial gland T, Mediastinal TT.
80	Tub. cow 41	5-17-04 1 cc.	6-7-04 2 cc.	7-14-04 3 cc.	8-11-04	......	Avian Culture P. I. 2	From birth	None, ...	12-30-05	Lungs, liver and Post-pharyngeal glands TT.
446, ...	Tub. Animal 182	3-13-06	4-28-06 2 cc.	6-25-06 4 cc.	......	......	B. I.	Partial exposure from birth	None, ...	Killed 11-5-08	Caseo-calcareous nodule in one mesenteric gland and one in axillary lymph gland. Inoc. of rabbits shows it to be bovine infection.
448, ...	Tub. cow 187	2-27-06	4-28-06 4 cc.	6-8-06 20 cc.	......	......	R. R.	Partial exposure from birth	None, ...	1-22-07	Lungs, bronchial glands, T. Mediastinal, TT.
450, ...	Tub. cow 316	3-15-06	5-26-06 4 cc.	8-29-06 20 cc.	......	......	R. R.	Partial exposure from birth	None, ...	Killed 11-6-08	Slight infection of posterior mediastinal gland, left Bronchial gland, posterior or tip of right lung and three mesenteric glands.
457, ...	Vaccinated Animal 13	3-24-06	4-28-06 2 cc.	6-8-06 4 cc.	7-20-06 6 cc. subcutaneously	......	B. I.	Partial exposure from birth	None, ...	Killed 11-6-08	Bronchial glands TT. Post-pharyngeal TT. Mesenteric TT.
498, ...	Animal 76	6-13-06	8-29-06 4 cc.	11-21-06 20 cc.	......	......	R. R.	Partial exposure from birth	None, ...	Killed 11-6-08	Post pharyngeal TT. Left bronchial, T. Liver T.

As controls for the above vaccinated calves there were 24 calves which were not injected with tubercle bacilli, but kept in a similar manner. A large number of these controls died during the progress of the work and on autopsy were found to be badly diseased, in many cases showing generalized tuberculosis. In no instance were any of them free from tuberculosis. In comparing the autopsy results of the vaccinated animals with the controls, there is a marked difference in the extent and distribution of the lesions. In the controls the lesions were more extensive, more widely distributed and distinctly progressive, while in the vaccinated animals they were dry, caseous-calcareous or calcareous, and in many instances surrounded by a firm fibrous capsule. The following table will show the origin of the control as well as the post-mortem findings.

TABLE OF CONTROL CALVES.

Animal No.	Dam.	Date of birth.	Date Exposed to Infection from Tubercular Cows.	Killed or Died.	Postmortem Findings.
57	Animal 20029, ...	....	From birth, ...	Died 12/30/05, ...	Generalized tuberculosis.
61	Animal 10, ...	....	From birth, ...	Died 11/18/05, ...	Tuberculosis of lungs TTTT. Mediastinal glands TTT. Post-pharyngeal glands. TTT. Tubercular abscess back of pharynx connecting with post pharyngeal gland.
81	Tub. cow 31, ...	6/3/04	From birth, ...	Died 10/14/06, ...	Generalized tuberculosis including udder.
90	Animal 16025, ..	6/29/04	From birth, ...	Killed 6/19/06, ...	Tuberculosis of lungs TT. Mesenteric glands T.
91	Animal 11, ...	6/29/04	From birth, ...	Killed 8/26/05, ..	Tuberculosis of lungs TT. Bronchial glands TT. Mediastinal glands, TTT.
122	Cow 45190, ...	9/3*04	From birth, ...	Killed 11/18/05, .	Lungs TT. Anterior medistinal TT. Left bronchial gland.
157	Cow 46, ...	10/17/04	From birth, ...	Killed 5/12/06, ..	General tuberculosis of lungs, bronchial glands, mediastinal gland, mesenteric glands, liver and right femur-tibia joint.
314	Animal 18, ...	4/8/05	From birth, ...	Died 12/10/07, ...	Acute tubercular pneumonia of lungs. A few old caseous lesions found in substance of lung tissue.
337	Animal 193, ...	4/16/05	From birth, ...	Died 11/8/07, ...	Tuberculosis of lungs TT. Mediastinal glands TTTT. Liver TT. Mesenteric glands T. Post-pharyngeal glands TTT.
407	Animal 20025, ..	11/28/05	Partial exposure from birth, ...	Killed 9/2/07, ...	Killed in moribund condition. General tuberculosis of lungs, pleura, bronchial and mediastinal glands, diaphragm, pericardium and post-pharyngeal glands. One gland in intermaxillary space caseous.
408	Cow 184, ...	12/8/05	Partial exposure from birth, ...	Killed 1/22/07, ..	Tuberculosis of lungs TT. Bronchial and mediastinal glands TT and left post-pharyngeal gland TT.
411	Animal 185, ...	2/4/06	Partial exposure from birth, ...	Killed 1/2/08, ...	General tuberculosis of mediastinal, bronchial and post-pharyngeal lymphatic glands.
413	Animal 11, ...	1/31/06	Partial exposure from birth, ...	Died 7/29/07, ...	General tuberculosis of lungs, bronchial and mediastinal glands, liver, portal lymphatic gland, peritoneum, omentum and several mesenteric glands. Also articulation of right fore knee.
414	Animal 4, ...	2/2/06	Partial exposure from birth, ...	Died 2/13/08, ...	Extensive tuberculosis of pleura; also lesions in one submaxillary gland.
415	Animal 15, ...	12/23/05	Partial exposure from birth, ...	Died 1/2/08, ...	General tuberculosis of lungs, bronchial glands, mediastinal glands, liver and portal lymphatic gland.
419	Animal 19, ...	2/6/06	Partial exposure from birth, ...	Died 5/11/06, ...	Cause of death pneumonia. Lesions of tuberculosis found in bronchial and mediastinal glands.
420	Animal 10, ...	12/26/05	Partial exposure from birth, ...	Died 5/3/06.	Cause of death, intestinal trouble. Lesion of active tuberculosis $\frac{3}{4}''$ in diameter found in left post pharyngeal gland.
422	Animal 22, ...	2/6/06	Partial exposure from birth, ...	Killed 5/12/06, ..	Animal was moribund when destroyed. Lungs in pneumonic condition. Lesions of tuberculosis found in the bronchial and mediastinal glands.
428	Animal 28, ...	2/15/06	Partial exposure from birth, ...	Died 1/2/08, ...	Tuberculosis of pleura TTTT, lungs TTT, pericardium TT, mediastinal and bronchial glands TTTT, liver TT, portal lymphatic gland T, and post-pharyngeal TT.
430	Animal 20, ...	2/21/06	Partial exposure from birth, ...	Died 1/2/08, ...	Tuberculosis of lungs T, mediastinal glands TT, bronchial TT, liver TT, spleen TT.
745	Animal 316, ...	3/3/07	Exposed after 12/27/07, ...	Killed 6/6/10, ...	An evenly distributed tuberculosis of lungs. Other organs normal.

TABLE OF CONTROL CALVES—Concluded.

Animal No.	Dam.	Date of birth.	Date Exposed to Infection from Tubercular Cows.	Killed or Died.	Postmortem Findings.
749	Animal 82, ...	3/19/07	Exposed after 1/5/08, ...	Killed 6/6/10,...	Tuberculosis of post-pharyngeal gland T, mediastinal glands TT, and lungs TTT.
757	Animal 79, ...	4/19/07	Exposed after 11/6/07, ...	Died 2/26/08,....	Death due to tympanites. Lesions of tuberculosis found in lungs and posterior mediastinal lymph gland.
780	Animal 279, ...	7/16/07	Exposed after 12/27/07, ...	Killed 2/13/08,..	Posterior mediastinal gland enlarged, on section caseous throughout. Portal lmyphatic gland slightly enlarged and on section caseo-calcareous.

PLATE VIII.—Pens in which immunized calves and controls were exposed to infection with tuberculous cows. Experiment 115 I.

PLATE IX.—The feeding of pigs upon the milk from immunized cows.

EXPERIMENT 115J.

The object of this experiment was to test upon young animals the immunization effect when used as food, of the milk of cows immunized against tuberculosis. Eight young pigs were selected for this purpose about eight weeks of age. Six of them were fed on milk from cows that had received ten vaccinations. The cows used for this purpose were the animals in Lot E, Experiment 115H. The feeding of the milk started on July 2, 1904, and was continued twice daily until April 12, 1905. All of the pigs, both the ones receiving the milk from immunized cows as well as the two controls, were fed during the month of October, 1904, 2 cc. of a standard suspension of Culture M, and in December of the same year they received four feedings of 5 cc. of the same culture. During the experiment the two control pigs were kept in an entirely separate building and were fed on milk from animals that had not been vaccinated. Early in March 1905 the six immunized pigs were given five feedings of 10 cc. each of a standard suspension of tubercle bacilli of Cultures H and L. These cultures were of bovine origin and known to be highly virulent for cattle. The two control pigs did not receive any feedings of these cultures.

On May 26th all of the animals were killed. Three of the immunized pigs were found to be free from tuberculosis, while the remaining three immunized animals showed small lesions in the Mesenteric Lymphatic Glands. In two of them only three of the Glands were involved, while in the third the lesions were more extensive, but confined entirely to the Lymphatic Glands of the Mesentery. At autopsy one of the control animals showed a caseous nodule in the sub-maxillary lymphatic gland, while in the other a careful post-mortem examination failed to reveal any lesions of tuberculosis. The absence of lesions in this animal as well as the slight lesion in the other control might be explained from the fact that these two control pigs were not given the five feedings of 10 cc. each of the suspension of bovine tubercle bacilli, as it was believed that the feedings of Culture M which were given during October and December, would be sufficient to infect them. It had been previously found by other experiments of the State Livestock Sanitary Board that pigs were about equally as susceptible to tubercle bacilli of the human type as they were to tubercle bacilli of the bovine type. It seems that the animals receiving the immunized milk manifested a certain degree of resistance for the bovine cultures H and L, of which they received five feedings of 10 cc. each. These cultures were proven to be virulent for pigs. One pig which received five feedings of 10 cc. each every other day from March 13th to March 22nd was killed on May 15th of the same year and found to contain rather extensive lesions of tuberculosis in the lungs, bronchial and mediastinal lymphatic glands, and in the lymphatic glands in intermaxillary space, which were enlarged to the size of an orange and filled with thick creamy pus. The spleen, liver and mesenteric lymphatic glands were also involved.

EXPERIMENT 115K.

In 1905 Pearson & Gilliland reported in the University of Pennsylvania Medical Bulletin an experiment upon "The Effect of Tuberculosis Vaccination upon Cattle infected with Tuberculosis." In that experiment twelve young calves were used between the ages of six to eight months. These animals were taken from a herd in which a large percentage of the animals had reacted to tuberculin including these twelve calves. The calves prior to the experiment were again tested with tuberculin and all responded. Six of the calves were treated and six kept as controls. Three of the treated calves were given seven intravenous injections of a standard suspension of tubercle bacilli, culture M. The other three treated calves were given subcutaneous injections of tuberculin at intervals of two to ten days. The injections of tuberculin were repeated until the hypersensitiveness of the animal had disappeared, after which they received an intravenous injection of a standard suspension of tubercle bacilli, culture M. Following each intravenous injection of living tubercle bacilli, the animal was again given tuberculin a number of times until its hypersensitiveness disappeared.

The control calves were given no treatment whatever, but were at all times kept with the six calves under treatment. At the time the calves died or were killed, there was a distinct difference in the extent and appearance of the lesions of the treated animals and those of the controls. Two of the control animals died in six months and nine months respectively from the beginning of the experiment, while all of the treated animals lived until the termination of the experiment, approximately one year nine months from the time it was started. The lesions in all the treated animals were quiescent and encapsulated, and the authors claimed at that time that the treatment to which the six animals were subjeced had the effect not only of keeping in check the progress of the tuberculous process, but in causing a distinct and in some cases a great retrogression of the lesions. They further state that the experiment was made on too small a number of cattle to permit of any conclusions or inferences as to the probable effect of similar treatment on older and more extensively diseased animals.

With this knowledge at hand another experiment was started with the object of determining to what extent lesions of tuberculosis of older cattle can be cured or retarded by similar treatment. For this purpose seven heifers were selected which had given a positive tuberculin reaction and had come from an extensively tubercular herd in Tioga County. Four of the animals were treated and three kept as controls. One of the treated animals, after having received three injections of tubercle bacilli and nine injections of tuberculin, died from a post-partum infection and therefore must be excluded from the experiment. The three remaining treated animals, 178-79-80, received the following injections of tubercle bacilli:

1905.

January 17th, injected in jugular vein, 3 cc. Standard Suspension Culture M.

February 21st, injected in jugular vein, 4½ cc. Standard Suspension Culture M.

March 21st, injected in jugular vein, 6 cc. Standard Suspension Culture M.

April 28th, injected in jugular vein, 7½ cc. Standard Suspension Culture M.

June 3rd, injected in jugular vein, 9 cc. Standard Suspension Culture M.

1906.

February 21st, injected in jugular vein, 6 cc. Standard Suspension Culture M.

These animals were given tuberculin subcutaneously following each intravenous injection until the hypersensitiveness to tuberculin disappeared. However, no tuberculin was given them following the last injection of tubercle bacilli on February 21, 1906. The initial dose of tuberculin following each injection of tubercle bacilli was 1 cc. and was increased ½ cc. at each test until the animal lost its hypersensitiveness to the same. The number of injections of tuberculin given throughout the treatment of the three calves consisted of three injections following the first, second and third inoculations of tubercle bacilli, four injections following the fourth injection of tubercle bacilli and seven injections following the fifth injection of tubercle bacilli. There was no tuberculin given following the last injection of tubercle bacilli. The animals gave typical thermal reactions following the injections of tuberculin after the first and second vaccinations. After that the hypersensitiveness appeared to diminish.

During the experiment the treated animals and controls were kept together and were exposed to a certain degree of infection by being housed in stables that had been used for tuberculous animals.

All of the animals excepting one were tuberculin-tested early in January, 1907, and gave a positive reaction. They were killed a few days later with the following results:

TREATED ANIMALS NOS. 178, 179 AND 180.

Animal No. 178 was killed January 10, 1907. Condition of animal good. In the principal lobe of the left lung there was a firm mass ½″ x ⅜″. On section this mass shows a thick gray outer wall with bands of white fibrous tissue extending across from wall to wall. Between these bands there is caseous material which can be removed completely, leaving a multilocular cyst-like lesion. The lung tissue surrounding this lesion was normal. In the right lung there are three lesions approximately ½″ in diameter, all of which are well encapsulated with thick fibrous tissue. Guinea pigs and rabbits inoculated from these lesions developed tuberculosis. Left bronchial lymphatic gland slightly enlarged and in its interior is a soft and caseated nodule surrounded by a capsule. The right bronchial gland is not enlarged, but on section found to contain two small caseous foci ⅛ and 3-16 of an inch respectively in diameter. One Post-pharyngeal Lymphatic Gland is nodulated and on section shows numerous caseated foci.

Animal No. 179. Killed January 2, 1907. Condition good. The lungs were well inflated with air and normal in appearance. On section nine encapsulated nodules from ½ to ¾ of an inch in diameter were found. Each nodule is surrounded by a firm capsule and is sharply separated from the adjoining healthy lung tissue except in one instance in which there is a fresh invasion of lung tissue adjacent to an encapsulated nodule. The new lesion is firm in texture and dark red in color. The area was about ½ x ¾ of an inch and contained small caseous points the size of a millet seed. The mesenteric glands contained a few points of dry caseation surrounded by a grayish capsule. All other organs, glands and membranes normal.

Animal No. 180. Killed January 10, 1907. Condition fair. In the right lung there was a nodulated area which on section shows numerous cavities. These cavities appear to communicate by rather dense fibrous tissue and contain a viscous fluid amber in color. Guinea pigs inoculated subcutaneously with an emulsion of this viscous material developed tuberculosis. Cultures made directly from the fluid in these cavities revealed the presence of the bacillus pyocyaneus. The right bronchial lymphatic gland is normal in size, but on section shows three caseo-calcareous nodules ⅛″ in diameter. In the left lung no lesions were found in the lung substance, but the pleura covering the same is very much thickened over the internal lobe. In the posterior mediastinal lymphatic gland there is one calcareous focus which is well encapsulated. All other glands, membranes, etc., normal.

CONTROL ANIMALS NOS. 181, 182 AND 183.

Animal No. 181. Killed January 10, 1907. Condition fair. The right costal pleura shows numerous dense fibrous growths as far as the sixth rib. The lungs contained a number of large masses varying in size up to 4 and 5″ in diameter. They fluctuate and on section showed the contents to be of soft caseous material. These masses are evenly distributed throughout the lung tissue. The pleura covering the lungs is thickened in many places and contains small nodules united by fibrous tissue. The Mediastinal and Bronchial Lymphatic Glands are enlarged and on section contained dry caseous material. The liver was extensively diseased as well as the Portal, Post-pharyngeal and Mesenteric Lymphatic Glands.

Animal No. 182. Killed January 10, 1907. Condition fair. In both lungs there was found a number of caseous masses varying in size up to 7″ x 2″. None of these lesions showed distinct encapsulation. The Bronchial Lymphatic Glands as well as the Mediastinal Lymphatic Glands contained caseous nodules. The anterior surface of the liver contained a caseous nodule 1-6″ in diameter. On section the liver showed two caseous masses. A few of the Mesenteric Lymphatic Glands as well as the Post-pharyngeal Lymphatic Glands were found to contain caseous foci.

Animal No. 183. Killed January 10, 1907. Good condition. Lungs contained a large number of firm caseous masses. The right Bronchial Lymphatic Gland and the Middle and Posterior Mediastinal Glands were caseous on section. The liver contained a number of caseous masses. Three of the Mesenteric Lymphatic Glands and one of the Post-pharyngeal Lymphatic Glands were slightly enlarged and on section showed a number of caseous foci.

In comparing these autopsies it is apparent that the control animals showed lesions more extensive and more widely distributed. The lesions in the treated animals appeared to be less active than those in the controls. However, none of the treated animals were free from tuberculosis, and the lesions found in them contained tubercle bacilli that were proven virulent for rabbits. It may be possible that the result between the treated animals and the control animals would have been more striking if the animals had been given more treatments and had been allowed to live for a longer time following the injections. These animals at the beginning of the experiment were older and probably more extensively diseased than the animals reported upon in 1905.

EXPERIMENT 115L.

The object of this experiment was to determine the extent to which cattle may be immunized against tuberculosis by frequent intravenous injections of small quantities of tubercle bacilli followed in some cases by subcutaneous injections of Original Tuberculin of Koch, and in other cases by Maragliano's Watery Extract of tubercle bacilli. Further information was to be gotten upon the value of von Bering's method of vaccination by the use of his Tuberkulose—Impfstoff für Rinder as found on the market. Two animals were to be used for this purpose.

The plan of the experiment which was followed as nearly as conditions would warrant, was to select ten head of yearling calves about the same size, weight and condition, and to prove that they are free from tuberculosis by the tuberculin test. The ten animals are to be divided according to size and condition into five subgroups of two animals each and to be known as Pairs A, B, C, D, and E, throughout the experiment.

The method of immunizing the animals was to be as follows:

Pair A are to receive four vaccinations of a Standard Suspension of Culture M. The first injection to consist of 2 cc. and each subsequent injection increased 1 cc.

Pair B are to receive an initial vaccination of 2½ cc. of a Standard Suspension of Culture M and to be followed in seven days with a subcutaneous injection of 0.5 cc. of tuberculin. The tuberculin injections are to be repeated at intervals of approximately five days, increasing the dose with each injection until the animal fails to respond. The animal is then to receive a second vaccination with a standard suspension of Culture M of a dosage slightly larger than the previous dose, and then followed by more subcutaneous injections of tuberculin. These vaccinations and injections of tuberculin are to be repeated until four vaccinations are given.

Pair C are to be vaccinated in a similar manner as Pair B, excepting Maragliano's Watery Extract of Tubercle Bacilli is to be used for the subcutaneous injections following the vaccinations in place of the Original Tuberculin of Koch.

Pair D are to be vaccinated with von Behring's Tuberkulose—Impfstoff für Rinder as purchased on the market and in accordance with the directions accompanying the same.

Pair E are to receive two vaccinations of a Standard Suspension of Culture M. The first vaccination to consist of the same quantity of tubercle bacilli as the first vaccination of Von Behring Vaccine, and the second vaccination to be of five times the initial dose. The interval between the vaccinations to be the same as recommended for Pair D.

The animals of each lot together with one control animal of a like size and free from tuberculosis, are to be infected by an intratracheal inoculation with a moderate dose of bovine tubercle bacilli four weeks after the last vaccination.

This experiment was started in December, 1904, and was carried out as near the original plan as above outlined as conditions would permit. The animals were carefully selected from the stockyards in Philadelphia and were subjected to a most careful tuberculin test.

The animals for Pair A consisted of a red heifer No. 147 and a black steer No. 149. These two animals received four vaccinations as follows:

1905.

January 13th, inoculated in jugular vein, 2 cc. Standard Suspension Culture M.

January 25th, inoculated in jugular vein, 3 cc. Standard Suspension Culture M.

February 4th, inoculated in jugular vein, 4 cc. Standard Suspension Culture M.

February 14th, inoculated in jugular vein, 5 cc. Standard Suspension Culture M.

On March 14th, four weeks following the last vaccination, these two animals with one control, No. 192, were inoculated intratracheally with 5 cc. of a Standard Suspension of Culture H. This Culture was of bovine origin and known to be highly virulent for cattle. Its virulence has been referred to earlier in this article.

All of these animals were killed approximately ten weeks following the date they were infected intratracheally, with the following results:

Red heifer No. 147. Killed June 3, 1905. Condition fair. The only lesions of tuberculosis found were in the Mesenteric Glands and at the point of inoculation. The lesion in the Mesenteric Lymph Gland is 1-3″ in diameter and filled with cheesy material. It did not have the macroscopic appearance of tuberculosis, but guinea pigs inoculated from the same developed the disease. At the point of infection there is a thickening on the outside of the Trachea about ¾″ in diameter. On section it was found to be composed principally of fibrous tissue. In a Tracheal Lymphatic Gland close to the point of inoculation there were found on histological examination several small areas of tuberculosis with giant cells and a small amount of caseation.

Black steer No. 149. Killed June 3, 1905. Condition good. This animal was normal in every respect except at the point of infection. Outside of the Trachea there was a large thickened mass 4″ x 2″ which was nodular and caseous. The mucous membrane of the Trachea opposite this lesion shows no evidence of tubercles, and it is possible that the infecting dose was not gotton into the Trachea, but discharged in the subcutaneous tissues around the same. The lesion afore-described was well confined and the surrounding Lymphatic Glands were not enlarged and showed no evidence of tuberculosis.

Red heifer No. 192. (Control) Killed June 3, 1905. Condition fair. Lungs, TTT. Bronchial Lymphatic Glands, TTT. Mediastinal Lymphatic Glands, TT. Post-pharyngeal Lymph Glands, TTT. Lymphatic Glands situated along the Trachea, TTTTT. At the point of infection there is a large nodule 1½″ x ½″ which contains yellow pus. The mucous membrane of the Trachea from the Larynx to the Lungs thickly studded with nodules varying in size from ¼″ to 1-16″ in diameter.

The animals for Pair B consisted of a red steer, No. 148, and a red heifer, No. 153. These two animals were given four vaccinations and each vaccination was followed by subcutaneous injections of tuberculin until the animal failed to respond. The following table will give in detail the vaccinations and the tuberculin injections.

1905.

January 13th, inoculated in jugular vein, 2½ cc. Standard Suspension Culture M.

January 24th, injected subcutaneously 0.5 cc. Original Tuberculin.

February 3rd, injected subcutaneously 1 cc. Original Tuberculin.

February 10th, injected subcutaneously 1.5 cc. Original Tuberculin.

February 17th, injected subcutaneously 2 cc. Original Tuberculin.

February 27th, injected subcutaneously 2.5 cc. Original Tuberculin.

March 9th, injected subcutaneously 3 cc. Original Tuberculin.

March 17th, inoculated in jugular vein, 4 cc. Standard Suspension Culture M.

March 24th, injected subcutaneously 0.5 cc. Original Tuberculin.

March 31st, injected subcutaneously 1 cc. Original Tuberculin.

April 7th, injected subcutaneously 1.5 cc. Original Tuberculin.

April 14th, injected subcutaneously 2 cc. Original Tuberculin.

April 27th, inoculated in jugular vein, 6 cc. Standard Suspension Culture M.

May 5th, injected subcutaneously 0.5 cc. Original Tuberculin.

May 12th, injected subcutaneously 1 cc. Original Tuberculin.

May 19th, injected subcutaneously 1.5 cc. Original Tuberculin.

May 26th, injected subcutaneously 2 cc. Original Tuberculin.

June 3rd, inoculated in jugular vein, 10 cc. Standard Suspension Culture M.

June 13th, injected subcutaneously 0.5 cc. Original Tuberculin.

June 20th, injected subcutaneously 1 cc. Original Tuberculin.

June 28th, injected subcutaneously 1.5 cc. Original Tuberculin.

July 17th, injected subcutaneously 3 cc. Original Tuberculin.

July 25th, injected subcutaneously 3.5 cc. Original Tuberculin.

On August 26th, approximately 11 weeks following the administration of the Vaccine or 4 weeks following the last injection of tuberculin, these two animals with control animal No. 357, were inoculated intratracheally with 7½ cc. of a Standard Suspension of bovine tubercle bacilli Culture H, this culture being the same culture as was used for Pair A.

These animals were killed during January 1906, approximately five months following the date of intratracheal infection, with the following results:

Red steer No. 148. Killed January 20, 1906. Lungs normal. All Bronchial and Mediastinal Lymphatic Glands normal. At the point of infection on the outside of the trachea there is a fibrous thickening ½″ in diameter, which on section shows a small quantity of pus surrounded by thick fibrous walls. Immediately above this point there was found loose in the connective tissue a mass about the size of an egg, which on section is found to contain yellow pus surrounded by a stout, hard, fibrous wall. The mucous membrane of the trachea at the point of infection is slightly thickened and contains a few yellow points which on pressure exude creamy pus. All other glands, organs, etc., normal.

Red heifer No. 153. Killed January 20, 1906. Condition fair. This animal was normal in every respect except the Trachea at the point of infection. The mucous membrane of the Trachea showed no tubercles except at the point in the median line on the anterior surface there is a nodule ⅛″ in diameter. On the outside of the Trachea opposite this point there is a thickening ¾″ in diameter, which on section is found to contain yellow caseous material surrounded by a dense fibrous wall. Guinea pigs inoculated from this material developed generalized tuberculosis.

Red and white heifer No. 357 (Control). Killed January 9, 1906. Condition fair. This animal had been suffering for some time prior with marked Dyspnoea with severe paroxysms of coughing. Lungs, TT. Bronchial Lymphatic Glands, TTT.

Mediastinal Lymphatic Glands, TT. Post-pharyngeal Lymphatic Glands, TT. All the Tracheal Lymphatic Glands from the Larynx to the Thoracic cavity are enlarged, hard and caseous. Some of these glands are 3″ in diameter. The mucous membrane of the Trachea from the Larynx down to the bifurcation of the same is covered with new growths varying in size from ¼″ to ⅓″ in diameter. On section these growths contained caseous material. The lumen of the Trachea at the point of infection was reduced to the diameter of the finger. On the outside of the Trachea opposite this point there was a large mass, which on section was filled with caseous material.

The animals for Pair C consisted of a red and white steer, No. 150, and a brindle bull, No. 151. These two animals were given four vaccinations and each vaccination was followed by subcutaneous injections of Maragliano's Watery Extract of Tubercle Bacilli. In brief, the treatment of these animals was the same as Pair B excepting they received Maragliano's Tubercle Bacilli Extract instead of Original Tuberculin prepared after the method of Koch. The exact date and doses of Vaccine administered for both animals, as well as the doses of Extract, were as follows:

1905.

January 13th, inoculated in jugular vein, 2½ cc. Standard Suspension Culture M.

January 24th, injected subcutaneously 0.5 cc. Watery Extract Tubercle Bacilli.

February 3rd, injected subcutaneously 1 cc. Watery Extract Tubercle Bacilli.

February 10th, injected subcutaneously 1½ cc. Watery Extract Tubercle Bacilli.

February 17th, injected subcutaneously 2 cc. Watery Extract Tubercle Bacilli.

February 27th, injected subcutaneously 2.5 cc. Watery Extract Tubercle Bacilli.

March 9th, injected subcutaneously 3 cc. Watery Extract Tubercle Bacilli.

March 17th, inoculated in jugular vein, 4 cc. Standard Suspension Culture M.

March 24th, injected subcutaneously 0.5 cc. Watery Extract Tubercle Bacilli.

March 31st, injected subcutaneously 1 cc. Watery Extract Tubercle Bacilli.

April 7th, injected subcutaneously 1.5 cc. Watery Extract Tubercle Bacilli.

April 14th, injected subcutaneously 2 cc. Watery Extract Tubercle Bacilli.

April 27th, injected in jugular vein, 6 cc. Standard Suspension Culture M.

May 5th, injected subcutaneously 0.5 cc. Watery Extract Tubercle Bacilli.

May 12th, injected subcutaneously 1 cc. Watery Extract Tubercle Bacilli.

May 19th, injected subcutaneously 1.5 cc. Watery Extract Tubercle Bacilli.

May 26th, injected subcutaneously 2 cc. Watery Extract Tubercle Bacilli.

June 3rd, inoculated in jugular vein, 10 cc. Standard Suspension Culture M.

June 13th, injected subcutaneously 0.5 cc. Watery Extract Tubercle Bacilli.

June 20th, injected subcutaneously 1 cc. Watery Extract Tubercle Bacilli.

June 28th, injected subcutaneously 1.5 cc. Watery Extract Tubercle Bacilli.

July 17th, injected subcutaneously 3 cc. Watery Extract Tubercle Bacilli.

July 25th, injected subcutaneously 3.5 cc. Watery Extract Tubercle Bacilli.

These two animals with a control, No. 358, were infected on August 26th, approximately 11 weeks following the last intravenous vaccination and four weeks following the last subcutaneous injection of tuberculin. The infecting dose consisted of 7½ cc. injected intratracheally of a standard suspension of bovine Tubercle Bacilli, Culture H. This suspension was the same as used for the infection of Pair B and a Control

These animals were killed with the following results:

Red and white steer No. 150. Killed January 20, 1906. Lungs normal. Mediastinal Glands normal. The left Bronchial Gland contained a caseo-calcareous nodule ¼″ in diameter. A small Lymph Gland close to the bifurcation of the Trachea on section contained a calcified point ⅛″ in diameter. Along the Trachea from the point of infection to the Thoracic cavity there were found three Lymph Glands, which on section contained caseo-calcareous points. Outside of the Trachea at the point of infection there is a thickened mass ¾″ in diameter and firmly adherent to the Trachea. On section it was found to contain pus. The mucous membrane of the Trachea contained a number of pinkish growths around the point of infection, and becoming fewer in number away from said points. These growths are 1-12 to 1-8″ in diameter, and on section some of the larger ones contain small points of caseation. None of the lesions appeared to be active.

Brindle bull No. 151. Killed January 22, 1906. Lungs normal. Mediastinal Lymphatic Glands normal. Bronchial Lymphatic Glands normal. Mucous membrane lining large Bronchi normal. The lower portion of the mucous membrane of Trachea contained a soft tubercle 1-12″ in diameter. At the point of intratracheal infection the mucous membrane is thickened on the anterior face. Immediately opposite this point the Trachea is greatly thickened. This point is about twice the usual thickness of the Tracheal wall and is composed of fibrous tissue, surrounding two small foci of caseation. On the outside and back of the Trachea there is a firm fibrous growth 1½″ long x ½″ in width. Around this mass there are two other nodules. The lesions indicate that a portion of the infecting dose was discharged in the subcutaneous tissue around the Trachea. Two Mesenteric Glands are filled with small areas of caseation. Post-pharyngeal Lymphatic Glands, TTT.

Red and white heifer No. 358 (Control). Killed November 18, 1905. Lungs, TT. Lymphatic Glands along the Trachea, TTTTT. Post-pharyngeal Lymphatic Glands, TTT. Bronchial Lymphatic Glands, TT. The mucous membrane of the Trachea is studded with soft red tubercles from the Larynx down to the Bronchi. There is a distinct thickening at the point of infection.

It will be noted that this control animal was killed much earlier than the two vaccinated animals and that the lesions in the same were more extensive and more progressive than in the treated animals.

The animals for Pair D consisted of roan steer, No. 146, and red heifer, No. 152. These two animals were vaccinated with von Behring's Tuberkulose-Impfstoff für Rinder in accordance with the directions for the administration of the same that accompanied the package. The dates of vaccination and the dose of Vaccine for both animals were as follows:

1905.

March 8th, inoculated in jugular vein, 0.004 grams von Behring Tuberkulose-Impfstoff für Rinder.

May 31st, inoculated in jugular vein, 0.02 grams von Behring Tuberkulose-Impfstoff für Rinder.

These two animals with their control, No. 359, were infected intratracheally on August 26th, approximately 2 3-4 months following the last vaccination with 7½ cc. of a Standard Suspension of bovine tubercle bacilli, Culture H. This same suspension was used to infect the calves in the two preceding pairs.

These three animals were killed approximately five months from the time of the intratracheal infection with the bovine tubercle bacilli, with the following results:

Animal No. 146. Killed January 20, 1906. The pleural surface of the lungs exhibited a number of tuberculous lesions, which were located principally at the posterior border of the lungs. Some of these lesions are ⅓″ in diameter. In the anterior lobe of the right lung there were found six firm tubercles, which on section had cheesy centers. The middle and posterior lobe of this lung contained a number of tuberculous foci. All of the Mediastinal Lymphatic Glands contained a number of caseous points. The mucous membrane of the Trachea contained a number of soft tubercles which on section were found to have caseous centers. These are distributed from the point of infection down to the lung tissue. Outside of the Trachea at the point of infection there are four nodules which on section are found to contain pus. One of the Tracheal Lymphatic Glands contained caseous material. Right Post-pharyngeal Gland contained a caseous focus.

Animal No. 152. Killed January 20, 1906. Both lungs contained scattered throughout all the lobes a large number of tubercles which have undergone caseation. The left Bronchial Gland is enlarged and on section is thickly studded with yellow points of caseation. Mediastinal Lymphatic Glands tuberculous, TTT. Lymphatic Glands of the Lung and Trachea tuberculous, TTT. The mucous membrane of the Trachea below the point of infection contains six or more nodulated elevations ⅓″ in diameter. On section these elevations are caseous. Outside of the Trachea at the point of infection there is a mass containing yellow caseous material. All other glands and organs normal.

Red heifer No. 359 (Control). Killed January 20, 1906. Lungs tuberculous, T. Bronchial Lymphatic Glands, TT. Mediastinal Lymphatic Glands, TTT. The mucous membrane of the upper portion of the Trachea contains two tubercles.

Outside of the Trachea at the point of infection there are several large abscesses containing soft caseous pus. Some of them are attached to the skin, while others are free in the subcutaneous tissue. The Lymphatic Glandes along the Trachea contain a number of caseous foci. From the mass outside of the Trachea it is believed that a portion of the infecting dose was discharged in the subcutaneous tissue.

The animals for Pair E consisted of a red heifer, No. 154, and a brown and white heifer, No. 193. These two animals were given two vaccinations of standard suspension of Culture M. The dosage of dried tubercle bacilli contained in the suspension was the same as the dosage for the preceding pair, which received the bovovaccine of von Behring. The dates and dosage of administration for this pair are as follows:

1905.

March 8th, inoculated in jugular vein, 0.004 grams of human tubercle bacilli Culture M. (Equal to 3.1 cc. Standard Suspension.)

May 31st, inoculated in jugular vein, 0.02 grams of human tubercle bacilli Culture M. (Equal to 16.4 cc. Standard Suspension.)

These two calves with a control, No. 360, were infected on August 26th in the same manner and with the same dose of bovine tubercle bacilli as for the preceding pairs B, C and D. These animals were all killed approximately five months following intratracheal infection, and the results of the autopsies are given as follows:

Red heifer No. 154. Killed January 20, 1906. Lungs and their associated Lymphatic Glands were normal. One Lymphatic Gland along the Trachea contained three areas of caseation varying in size from 1-12 to 1-6″ in diameter. One Post-pharyngeal Gland shows a small caseous point well encapsulated. The mucous membrane of the Trachea contains but one small elevation at the point of inoculation. Outside of the Trachea at this point is found an elevation ¼″ in diameter, firmly attached to the Trachea and on section contains yellow pus surrounded by a thick fibrous wall. All other organs, glands, etc., normal.

Animal No. 193. Killed January 20, 1906. Condition fair. The left lung on section shows a number of firm grayish tubercles which are all sharply encapsulated. A number of these are quite hard and calcified and are surrounded by a grayish capsule. The lung tissue surrounding these tubercles is entirely normal in appearance. Both Bronchial Lymphatic Glands contained caseous foci on section. The Mediastinal Lymphatic Glands show a number of caseo-calcareous points. The mucous membrane of the Trachea was normal except at the point of infection, where there is a slight elevation 1-6″ x 1-8″. There is another small tubercle directly opposite this lesion. On the outside of the Trachea at the point of inoculation there is a firm mass beneath the skin 1″ in diameter, which on section was found to consist of a greenish yellow pus. The Post-pharyngeal Lymphatic Glands were slightly enlarged and contained caseous foci. Three of the Mesenteric Lymphatic Glands were in a similar condition. One Lymphatic Gland along the Trachea was enlarged and on section showed a caseous mass 1″ in diameter. Guinea pigs inoculated from lung tissue developed tuberculosis. Rabbits and guinea pigs inoculated from caseous material found in Lymphatic Glands died of generalized tuberculosis.

Red and white heifer No. 360 (Control). Killed January 20, 1906. Left lung shows four nodules close to the pleural surface containing caseous centers. In the right lung there are four similar nodules which have undergone caseation. The Bronchial Lymphatic Glands were enlarged and caseous. All of the Mediastinal Lymphatic Glands enlarged and filled with caseous material. The Lymphatic Glands along the Trachea in a similar condition. The mucous membrane of the Trachea contains many new growths as large as ½″ in diameter, which on section are found to be caseous. Some of the Lymphatic Glands around the point of inoculation were 3½″ in diameter. Post-pharyngeal Glands greatly enlarged and tuberculous to the extent of TTTT. Mesenteric Lymphatic Glands, about 25 in number, were enlarged and contained caseous material.

At the time the animals in Pairs B, C, D and E with their controls were infected with 7½ cc. of a bovine culture of tubercle bacilli, another normal animal was infected in the same manner with 5 cc. of the same suspension. This was done believing that the 7½ cc. was possibly too large an infecting dose. This animal was inoculated intratracheally on August 26th on the same day as the other animals, and was killed five months later on January 20, 1906, with the following results:

Control animal No. 194. Left lung was of uniform texture except firm hard nodules could be felt underneath the serous membrane of the posterior lobe. They were yellowish in color and had caseous centers. The right lung contained a number of smaller nodules. Both Bronchial Lymphatic Glands contained several caseous foci. The anterior and middle Mediastinal Lymphatic Glands contained points of caseation. All of the Lymphatic Glands along the Trachea from the Pharynx to the Lungs were enlarged and contained caseous material. A number of the Mesenteric Lymphatic Glands contained caseous centers. One gland was 1½″ in diameter. Others were slightly enlarged.

In January 1906 the animals that had not been previously killed were tested with tuberculin and all of them gave a typical reaction except the two treated animals of Pair B, No. 148 and No. 153. The results do not permit of any definite conclusions being drawn between the various methods of immunizing the animals. However, all of the animals vaccinated by the five different methods showed a greater degree of resistance against infection than the controls. It is believed that the infecting dose of 7½ cc. excepting for Pair A was entirely too heavy to make any comparison of the different methods of vaccinating. It is the writer's belief that the method of intratracheal infection is not desirable for the reason that the animal may expel by coughing some of the infecting dose at the time it is administered. Again, there is danger of not getting all of it into the Trachea. Any method of infection by which Trauma is produced is not satisfactory.

EXPERIMENT 115 M.

The purpose of this experiment was to determine whether immunity, and if so, how much immunity could be conferred by treating calves with mild cultures of tubercle bacilli administered subcutaneously.

The cultures selected for this purpose were Culture BI and Culture M. The latter Culture was used in the preparation of the Vaccine for the previous experiment and has been heretofore described. Culture BI was obtained from the Bureau of Animal Industry and was supposed to be of human origin. It has been cultivated on artificial media for a number of years, and it was found to have very little virulence for experimental animals. Four guinea pigs inoculated with 3 to 4 cc. of a standard suspension failed to develop tuberculosis excepting one pig showed tuberculosis of the liver and omentum. A calf inoculated subcutaneously with 50 cc. of a standard suspension was killed five months later and found to contain no lesions of tuberculosis. The tissues around the points of the subcutaneous inoculation were indurated and thickened. The Lymphatic Glands around this point were normal. Monkeys inoculated subcutaneously with a very large dose of this culture developed a mild tuberculosis. It was therefore assumed that the culture had a very low degree of virulence and would be well adaptable for the first vaccination in this experiment.

The plan of the experiment was to obtain eight calves of approximately the same age and weight and to give five of them two subcutaneous vaccinations and the other three to be kept as controls. The first vaccination to consist of 5 cc. of a standard suspension of

Culture BI and followed in six weeks with the same dose of a standard suspension of Culture M.

Eight calves were purchased at the stockyards in Philadelphia for this purpose, and after they were tuberculin-tested and failed to react, five of them, No. 511, No. 512, No. 515, No. 516, and No. 519 were given two subcutaneous vaccinations, as follows:

1906.

July 9th, inoculated back of Scapula with 5 cc. of Standard Suspension of Culture BI.

August 25th, inoculated subcutaneously at median line of neck with 5 cc. of Standard Suspension of Culture M.

These five animals with their three controls, No. 508, No. 510 and No. 514, were kept under similar conditions and daily temperature measurements were taken and recorded. During December 1906, or approximately three months following the last vaccination, the entire eight animals were infected with virulent bovine tubercle bacilli, as follows:

December 4th, inoculated intratracheally, 3 cc. Standard Suspension Culture H.

December 11th, fed on bran 20 cc. Standard Suspension Culture H.

The first vaccination of the five animals produced no swelling at the point of injection except in animal No. 511, which showed a slight swelling on July 15th, but disappeared within a few days. However, the vaccination with Culture M on August 25th produced a distinct swelling at the point of injection in all of the vaccinated animals. These swellings remained for some months and at the time of infection with the bovine tubercle bacilli all of the vaccinated animals showed a slight swelling about 1″x3-4″ at the point of the second vaccination. In one animal, No. 115, however, this swelling was scarcely noticeable.

All of the animals, both the vaccinated and controls, developed hard lumps over the Trachea at the point of infection with the bovine tubercle bacilli. These lumps were evidently due to a small portion of the suspension being gotten underneath the skin.

All of the animals either died or were killed within ten months following the date of infection with the bovine tubercle bacilli, with the following results:

VACCINATED ANIMALS.

Animal No. 511. Killed June 7, 1907. Condition good. In the left lung two lesions were found about ½″ x ½″ in diameter. Histological examination of these lesions proved them to be nodules of early tuberculosis surrounded by a fibrous capsule. Right lung normal except for a nodule ¼″ in diameter which was firm and hard. Right Bronchial Gland on section showed 6 or 8 nodules of beginning caseation. Liver and Spleen showed a number of nodules of beginning caseation. A few lesions found in the Mesenteric Lymphatic Glands. A hard caseous mass was found at the point of infection in the Trachea. The mucous membrane of the Trachea contained several nodules both above and below the point of injection.

Animal No. 512. Killed June 7, 1907. Condition fair. The only lesions of tuberculosis found in this animal were at the point of infection into the Trachea, where there was a hard mass 1¼″ in diameter filled with yellow pus. This mass connected with a similar mass underneath the skin 2″ to 3″ in diameter which contained pus. Rabbits inoculated from this pus developed tuberculosis. One pre-scapular lymph gland was enlarged and found to contain pus. It is believed that a greater portion of the infecting dose in this animal did not enter the Trachea.

Animal No. 515. Died March 10, 1907, from intestinal trouble. This animal was in fair condition, but had not been eating well for some weeks. Both lungs congested, but showed no evidence of tuberculosis. At the point of infection into the Trachea there was a hard non-fluctuating lump 1″ in diameter. A lymph gland situated below this lump was enlarged and contained caseous foci. Inside the Trachea the mucous membrane contained about ten elevations which were evidently the beginning tubercles, though they had not gone on to the stage of caseation.

Animal No. 516. Killed June 7, 1907. Condition good. The left costal pleura is studded with miliary tubercles, the right costal pleura being normal. Pleural surface of the left lung contained many nodules varying in size from ⅛″ to ½″ in diameter. These nodules were more prominent on the surface of the anterior and middle lobes than on the posterior lobe. Section of lung showed a number of caseous nodules ¼″ in diameter surrounded by a capsule. Left Bronchial Lymph Gland contained several small nodules. Right lung normal. Mediastinal Lymphatic Glands normal. Mucous membrane of Trachea normal. All other organs, glands, membranes, etc., normal.

Animal No. 519. Died June 2, 1907. Cause of death not given in autopsy. The only lesions of tuberculosis found in this animal was on the surface of both lungs, where there were found many clusters of miliary tubercles spread rather evenly over the lung pleura, as well as over the costal pleura. The tubercles had not undergone caseation. There was no thickening or tissue reaction at the point of infection. All other glands, membranes, etc., normal.

CONTROL ANIMALS.

Animal No. 508. Killed June 7, 1907. Condition good. The pleura on the right side contained three firm nodules ½″ in diameter. In the substance of the left lung there are two tuberculous areas, one ¼″ in diameter and the other slightly larger, which on section show caseation. One Mediastinal Lymph Gland contained a few points of caseation the size of a pin-head. In the right lung there were two distinct tuberculous lesions, one 2″ in diameter, which on section showed eight or ten caseous nodules ¼″ in diameter. The other one was found in the posterior lobe ¾″ in diameter and contained caseous points the size of a pin-head. Post-pharyngeal Lymphatic Glands are both enlarged and show areas of early tuberculosis. At the point of infection there was a mass 1″ in diameter which contained caseous material. A number of the Lymphatic Glands along the Trachea were enlarged and tuberculous. The mucous membrane of the Trachea at point of infection contained a nodule ¾″ in diameter. A number of other smaller nodules were found below the point of infection.

Animal No 510. Killed June 7, 1907. Tuberculosis found in the lungs to the extent of TT. Mediastinal Lymphatic Glands, TT. At the point of infection into the Trachea there was a fluctuating mass 1″ in diameter containing creamy pus. There was a second mass below this one of the same character. A number of the Lymphatic Glands of the Trachea enlarged and caseous. The right pre-scapular Lymphatic Gland caseous. Mucous membrane of the Trachea at point of inoculation contained a nodule ⅛″ in diameter. Left Post-pharyngeal Lymphatic Gland enlarged and caseous.

Animal No. 514. Killed June 7, 1907. Tuberculosis found in the Mediastinal Glands to the extent of TT. The Trachea at point of inoculation shows a tubercular mass ¾″ x 1½″ with several smaller masses surrounding it. A number of the Lymphatic Glands along the Trachea enlarged and caseous. The Pre-scapular Lymphatic Glands slightly enlarged, but showed no distinct points of caseation. All other glands, membranes, etc., normal.

In analyzing this experiment it would seem that there was not a marked difference between the lesions found in the immunized animals and those found in the controls. The control calves gained in weight during the experiment an average of 182 lbs., while the immunized calves gained on an average of only 142 2-5 lbs. When this experiment is compared with former experiments in which an intravenous method of vaccination was used, we are led to believe that the subcutaneous method of vaccination is not as efficient as the intravenous method. It is possible that the virulence of the Culture BI was so low that it did not have much immunizing value. It is quite evident from the experiment following this one that the infecting dose of 3 cc. intratracheally and 20 cc. by feeding of bovine tubercle bacilli was entirely too large to be able to draw any comparisons.

EXPERIMENT 115 N.

This experiment was started for the purpose of comparing two principles in vaccination against tuberculosis, i. e., as to whether more immunity is produced by a gradual increase of dosage with long intervals between the same than by the inter-action of a small dose of Vaccine on a previously administered large one.

The plan of the experiment was to vaccinate ten healthy calves of nearly the same size, age and weight with Culture M, as follows:

Calf No. 1 vaccinate 2 cc. 6 weeks vaccinate 2 cc.

Calf No. 2 vaccinate 2 cc. 6 weeks vaccinate 4 cc. 6 weeks vaccinate 6 cc.

Calf No. 3 vaccinate 2 cc. 8 weeks vaccinate 10 cc.

Calf No. 4 vaccinate 8 cc. 6 weeks vaccinate 1 cc.

Calf No. 5 vaccinate 12 cc. 6 weeks vaccinate 1 cc.

Calf No. 6 vaccinate 15 cc. 8 weeks vaccinate 1 cc.

Calf No. 7 vaccinate 10 cc. Tuberculin weekly until reaction ceases.

Calf No. 8 vaccinate 10 cc. Tuberculin weekly until reaction ceases.

Calf No. 9 vaccinate 10 cc. Tuberculin at 3 day intervals for 3 weeks.

Calf No. 10 vaccinate 10 cc. Tuberculin at 3 day intervals for 3 weeks.

The calves are to be kept in a similar manner and daily temperature measurements are to be taken and recorded. All of the animals are to be later infected intravenously with an injection of bovine tubercle bacilli that will kill unvaccinated calves in six to eight weeks.

The experiment was carried out as near the above outline as possible under the existing conditions. The exact treatment and results of the autopsy of each calf is as follows:

Calf No. 1 (Herd No. 683). Age about six months.

VACCINATIONS.

1906.

December 15th, injected in jugular vein, 2 cc. Standard Suspension Culture M.

1907.

January 25th, injected in jugular vein, 2 cc. Standard Suspension Culture M.

INFECTION.

1907.

March 22nd, injected intravenously 1.5 cc. Standard Suspension of Bovine Tubercle Bacilli, Culture H.

AUTOPSY.

Killed June 24, 1907. Condition good. On the inner surface of the left lung there were many yellow nodules of tuberculosis. These nodules are about ¼″ in diameter. On section these nodules are found to be scattered throughout the lung tissue. The nodules are distinct and separate and in no place do they run together to form a large caseous mass. The right lung is in a similar condition. Both Bronchial Glands contain several foci of caseation, varying in size from ⅛″ to ¼″. Mediastinal Lymphatic Glands show a number of caseous foci on section. All other glands and organs normal.

Calf No. 2 (Herd No. 690). Age about seven months.

VACCINATIONS.

1906.

December 15th, injected in jugular vein, 2 cc. Standard Suspension Culture M.

1907.

January 25th, injected in jugular vein, 4 cc. Standard Suspension Culture M.

March 7th, injected in jugular vein, 6 cc. Standard Suspension Culture M.

This animal gave a typical tuberculin reaction following the second and third vaccinations.

INFECTION.

1907.

May 1st, injected intravenously 1.5 cc. Standard Suspension of Bovine Tubercle Bacilli, Culture H.

AUTOPSY.

Killed August 1, 1907. Condition good. No macroscopic lesions of tuberculosis could be found in any portion of the carcass excepting one suspicious area in the anterior mediastinal gland and one area in the lung tissue. Guinea pigs inoculated from these suspicious points developed tuberculosis.

Calf No. 3. (Herd No. 693). Age about six months.

VACCINATIONS.

1906.

December 15th, injected in jugular vein, 2 cc. Standard Suspension Culture M.

1907.

February 11th, injected in jugular vein, 10 cc. Standard Suspension Culture M.

This animal gave a typical thermal reaction following the second vaccination, but no reaction following the first vaccination.

INFECTION.

1907.

April 8th, injected intravenously 1.5 cc. Standard Suspension of Bovine Tubercle Bacilli, Culture H.

AUTOPSY.

Killed July 11, 1907. Condition fair. The only lesion of tuberculosis found in this animal was in the tip of the right lung a nodule $\frac{1}{3}''$ in diameter. The nodule has a thick gray wall and a caseous center the size of a pin-head. The wall was unusually thick and constituted the whole mass of the nodule excepting the pin head center. At the point of infection in the jugular furrow there was a swelling the size of an egg that contains thick pus. This mass is not attached to the vein and no doubt was caused by some of the infecting dose being discharged outside of the vein.

Calf No. 4. (Herd No. 695). Age about seven months.

VACCINATIONS.

1906.

December 15th, injected in jugular vein, 8 cc. Standard Suspension Culture M.

1907.

January 25th, injected in jugular vein, 1 cc. Standard Suspension Culture M.

This animal gave a positive thermal reaction following both the first and second vaccinations.

INFECTION.

1907.

March 22nd, injected intravenously 1.5 cc. Standard Suspension Bovine Tubercle Bacilli, Culture H.

AUTOPSY.

Killed June 24, 1907. Condition good. Lungs normal excepting for two suspicious nodules $\frac{1}{4}''$ in diameter. Anterior Mediastinal Gland shows one nodule at periphery which has undergone caseation. Liver showed three or four yellow areas the size of a pin-head. A lymph gland along the jugular vein below the Larynx is enlarged and on section contains many points of caseation. At the point of inoculation of the Vaccine in the jugular furrow there is a mass $3''$ long x $\frac{3}{4}''$ in diameter, which on section contains yellow creamy pus. Guinea pigs inoculated from this pus developed tuberculosis.

Calf No. 5. (Herd No. 696). Age about seven months.

VACCINATIONS.

1906.

December 15th, injected in jugular vein, 12 cc. Standard Suspension Culture M.

1907.

January 25th, injected in jugular vein, 1 cc. Standard Suspension Culture M.

This animal gave a distinct thermal reaction following both the first and second vaccinations.

INFECTION.

1907.

March 22nd, injected intravenously 1.5 cc. Standard Suspension Bovine Tubercle Bacilli, Culture H.

AUTOPSY.

Killed June 24, 1907. Condition good. In the left lung at the endge of the principal lobe there were found three or four nodules $\frac{1}{4}$″ in diameter. No other lesions found except two Lymph Glands in the jugular furrow, one of which was greatly enlarged and had undergone caseation. The other gland was about 1″ in diameter and on section showed caseous material well encapsulated.

Calf No. 6. (Herd No. 682). Age about eight months.

VACCINATIONS.

1906.

December 15th, injected in jugular vein, 15 cc. Standard Suspension Culture M.

1907.

February 11th, injected in jugular vein, 1 cc. Standard Suspension Culture M.

This animal gave a distinct thermal reaction following both vaccinations.

INFECTION.

1907.

April 8th, injected intravenously 1.5 cc. Standard Suspension Bovine Tubercle Bacilli, Culture H.

AUTOPSY.

Killed July 7, 1907. Condition poor. At the point of infection there is a small swelling $\frac{3}{4}$″ in diameter. On section it is found to contain soft yellow caseous material. The wall of the vein is not involved. In the main lobe of the right lung there is found a yellow nodule 3-16″ in diameter. The costal pleura at the top of the same lobe shows three or four similar lesions. Right Bronchial Gland showed on section numerous dense yellow points of uniform texture, which on histological examination proved to be typical tubercles with caseation and giant cells. In the left lung there were found four or five nodules $\frac{1}{8}$″ to 3-16″ in diameter, most of them just below the pleura. On section they showed a firm wall with soft yellow centers. Heart muscles showed upon histological examination a sub-pericardial fibro-tuberculous nodule. Liver showed points of fibro caseous tuberculosis. Mediastinal Lymphatic Glands and Portal Lymphatic Glands show points of typical tuberculosis. One kidney showed an area of tuberculosis with round cell infiltration. Rabbits and guinea pigs inoculated from the lesions of this animal developed tuberculosis.

Calf No. 7. (Herd No. 688). Age about seven months.

TREATMENTS.

1906.

December 15th, injected in jugular vein, 10 cc. Standard Suspension Culture M.

1907.

January 21st, injected subcutaneously 0.4 cc. Original Tuberculin.

February 6, injected subcutaneously 0.4 cc. Original Tuberculin.

February 19th, injected subcutaneously 0.4 cc. Original Tuberculin.

February 27th, injected subcutaneously 0.6 cc. Original Tuberculin.

This animal did not give a very distinct thermal reaction following the intravenous injection of Vaccine, but gave a distinct reaction following the injection of tuberculin on January 21st and February 6th, and no reaction following the injections of tuberculin on February 19th and February 27th.

INFECTION.

1907.

May 1st, injected intravenously 1.5 cc. Standard Suspension Bovine Tubercle Bacilli, Culture H.

AUTOPSY.

Killed August 1, 1907. Condition good. In the left lung there is a typical tuberculous lesion in the tip of the posterior lobe ¼″ in diameter. Both Bronchial Lymphatic Glands showed a number of yellow dense nodules about ⅛″ in diameter. One of the Mediastinal Lymphatic Glands on histological examination showed small areas of early tuberculosis with slight caseation. Two rabbits inoculated from the emulsion of this Gland developed tuberculosis in six weeks. Other organs normal.

Calf No. 8. (Herd No. 694). Age about eight months.

TREATMENTS.

1906.

December 15th, injected in jugular vein, 10 cc. Standard Suspension Culture M.

1907.

January 21st, injected subcutaneously 0.4 cc. Original Tuberculin.

February 6th, injected subcutaneously 0.4 cc. Original Tuberculin.

February 19th, injected subcutaneously 0.4 cc. Original Tuberculin.

February 27th, injected subcutaneously 0.6 cc. Original Tuberculin.

This animal gave no reaction following the vaccination on December 15th, but gave a distinct thermal reaction following the injections of tuberculin of January 21st and February 6th, but no reaction following the last two tuberculin injections.

INFECTION.

1907.

May 1st, injected intravenously 1.5 cc. Standard Suspension Bovine Tubercle Bacilli, Culture H.

AUTOPSY.

Died June 7, 1907. The animal had been depressed for a week prior to death and showed labored breathing and coughing. Lungs showed acute miliary tuberculosis. Bronchial and Mediastinal Lymphatic Glands enlarged and congested. Post-Pharyngeal Lymphatic Glands greatly congested and on section one showed very small points of early caseation. Guinea pigs and rabbits inoculated from an emulsion of a Mediastinal Gland died of Tuberculosis in three weeks' time.

Calf No. 9. (Herd No. 686). Age about eight months.

TREATMENTS.

1906.

December 21st, injected in jugular vein, 10 cc. Standard Suspension Culture M.

December 26th, injected subcutaneously 0.4 cc. Original Tuberculin.

December 30th, injected subcutaneously 0.5 cc. Original Tuberculin.

1907.

January 3rd, injected subcutaneously 0.6 cc. Original Tuberculin.

January 7th, injected subcutaneously 0.8 cc. Original Tuberculin.

This animal gave a distinct thermal reaction following all of the injections.

INFECTION.

1907.

March 7th, injected intravenously 1.5 cc. Standard Suspension Bovine Tubercle Bacilli, Culture H.

AUTOPSY.

Killed June 4, 1907. Condition good. No lesions of tuberculosis in lungs excepting a suspicious nodule ⅛″ in diameter in the principal lobe of the left lung. It did not have the appearance of tuberculosis. Microscopic examination of scrapings from incised surface of lung failed to reveal any tubercle bacilli. Mediastinal Lymph Glands on section showed numerous yellow points of caseation located principally at the periphery of the Gland. Guinea pigs inoculated from these points developed tuberculosis. The Spleen upon histological examination contained one area partly circumscribed with a fibrous capsule and showed early caseation. Bronchial Lymphatic Glands upon microscopic examination showed areas of typical tuberculosis with numerous giant cells, slight caseation and some calcification. Post-pharyngeal Lymphatic Glands in similar condition. Histological examination from other organs of this animal failed to reveal any evidence of tuberculosis.

Calf No. 10. (Herd No. 687). Age about seven months.

TREATMENTS.

1906.

December 21st, injected in jugular vein, 10 cc. Standard Suspension Culture M.

December 26th, injected subcutaneously 0.4 cc. Original Tuberculin.

December 30th, injected subcutaneously 0.5 cc. Original Tuberculin.

1907.

January 3rd, injected subcutaneously 0.6 cc. Original Tuberculin.

January 7th, injected subcutaneously 0.8 cc. Original Tuberculin.

This animal gave a thermal reaction following all of the tuberculin injections, as well as following the first intravenous vaccination with Culture M.

INFECTION.

1907.

March 7th, injected intravenously 1.5 cc. Standard Suspension Bovine Tubercle Bacilli, Culture H.

AUTOPSY.

Killed June 4, 1907. Condition good. The right lung in the anterior lobe showed a large number of firm nodules which on section were found to be caseous. Guinea pigs inoculated from this lesion developed tuberculosis. Middle lobe is also tuberculous. Other portions of the lung normal. Left Bronchial Lymphatic Gland is normal in size and on section showed no distinct lesions of tuberculosis. However, guinea pigs inoculated from an emulsion of the same developed the disease. No other distinct lesions of tuberculosis found in this animal.

All of the animals in this experiment were proven to be free from tuberculosis by the tuberculin test before vaccination.

In order to determine the virulence of the culture used for infecting these control animals, two animals were inoculated intravenously with different doses. One animal received 7 cc. of a standard suspension and died of general tuberculosis in thirty days. The second animal received 3 cc. intravenously of the same suspension and died in twenty-seven days. Upon this information it was decided that a dose of 1.5 cc. of this culture would be sufficiently strong to kill a calf in six to eight weeks, and therefore this dose was selected for the infecting of the calves in this experiment.

EXPERIMENT 115 O.

The object of this experiment was to determine whether immunity to tuberculosis could be produced quickly by small daily vaccinations over a short period of time.

The general method was to vaccinate with a small dose each day for different periods and then to test the immunity by infecting one-half of the vaccinated animals the day following the last vaccination and the other half of the vaccinated animals eight weeks after the last vaccination.

Eight calves ranging in age from seven to eight months were selected for this purpose and divided into four lots of two animals each and known as Pair A, B, C and D.

The animals of Pair A were given an intravenous vaccination of 1 cc. of a standard suspension of Culture M each day for four days. The animals of Pair B were treated in a like manner each day for eight days, those of Pair C for 12 days and those of Pair D for 20 days. In other words, Pair A received four vaccinations, Pair B eight vaccinations, Pair C 12 vaccinations and Pair D 20 vaccinations.

One animal of each pair was infected the day following the last vaccination by an *intravenous* injection of 1½ cc. of a standard suspension of virulent bovine tubercle bacilli, Culture H. The remaining animal of each pair was infected with the same dose and in a similar manner approximately eight weeks following its last vaccination.

The following table will show in detail the date and dose of Vaccine as well as the time of infection and results of the autopsies:

TABLE OF ANIMALS EXPERIMENT 115 O.

Pair	Herd No.	Vaccinations.	Infection.	Killed or Died.	Autopsy.
AI	703	1 cc. daily for four days, Jan. 7th to 10th, inclusive.	1½ cc. intravenously bovine tubercle bacilli January 11th.	Died Feb. 1,	Tuberculous pneumonia. Lymphatic glands enlarged and congested.
AII	702	1 cc. daily for four days, Jan. 7th to 10th, inclusive.	1½ cc. intravenously bovine tubercle bacilli March 7th.	Killed June 7,	Pleura contained many miliary tubercles. Lungs and all lymphatic glands normal.
BI	692	1 cc. daily for eight days, Jan. 3rd to Jan. 10th, inclusive.	1½ cc. intravenously bovine tubercle bacilli January 11th.	Died Jan. 30,	Tuberculous pneumonia, with enlarged and congested lymphatic glands.
BII	701	1 cc. daily for eight days, Jan. 3rd to Jan. 10th, inclusive.	1½ cc. intravenously bovine tubercle bacilli March 7th.	Killed June 7,	A few hard nodules on pleura of right lung. A few congested areas in lungs and mediastinal gland. No macroscopic lesions. Guinea pigs inoculated from lung and gland developed tuberculosis.
CI	684	1 cc. daily for twelve days. Dec. 30th to Jan. 10th, inclusive.	1½ cc. intravenously bovine tubercle bacilli January 11th.	Died Jan. 29,	Tuberculous pneumonia. Smears made from lungs and glands showed many tubercle bacilli.
CII	685	1 cc. daily for twelve days, Dec. 30th to Jan. 10th, inclusive.	1½ cc. intravenously bovine tubercle bacilli March 7th.	Killed June 4,	Many early progressive tubercles on pleura. A number of nodules in lungs, associated lymphatic glands, liver, pericardium and portal gland.
DI	689	1 cc. daily for twenty days, Dec. 21st to Jan. 10th, inclusive.	1½ cc. intravenously bovine tubercle bacilli January 11th.	Died Feb. 20,	Miliary tuberculosis of lungs. Lymphatic glands enlarged and greatly congested.
DII	691	1 cc. daily for twenty days, Dec. 21st to Jan. 10th, inclusive.	1½ cc. intravenously bovine tubercle bacilli March 7th.	Killed June 7,	Lungs normal. Mediastinal and mesenteric lymphatic glands contain lesions of tuberculosis. Other organs normal.
Control	704	Control not vaccinated, ...	1½ cc. intravenously bovine tubercle bacilli January 11th.	Died Feb. 11,	Tuberculous pneumonia with early miliary tubercles. Enlarged and congested lymphatic glands.

It will be noted that three of the animals that were infected the day following the last dose of Vaccine, died within twenty days, while the fourth animal lived forty days, making the average length of life 24½ days. The control animal infected with the same dose lived 32 days. It is clearly shown that the calves immediately following the vaccinations possessed no immunity whatever. In fact, showed a less resistance to the infecting dose than the control animals.

The four animals that were infected eight weeks following the vaccinations showed a marked degree of resistance as compared to the control. However, all of them contained lesions of tuberculosis, but none of them had sufficient lesions to cause death within three months from the time of infection. In these four animals the lung tissue was comparatively free from nodules. The pleura seemed to be most badly affected. It is to be regretted that the infecting dose of bovine tubercle bacilli was so large. It is my belief that if this dose had been one-third the amount, the four calves that were not infected until eight weeks following the vaccination would have clearly resisted all infection.

This same experiment was carried out on four lots of rabbits of four animals each. One lot received four vaccinations. Another lot eight vaccinations, another lot twelve vaccinations and the remaining lot twenty-one vaccinations. Two rabbits of each lot were infected the day following the vaccinations and the other two animals were not infected until eight weeks following the vaccinations. Again, in this case the infecting dose was entirely too large, being one-third of the dose given the calves. The eight rabbits that were infected the day following the vaccinations lived on an average of 28 3-8 days, while the eight rabbits that were not infected until eight weeks following the vaccinations, lived on an average of 30 2-3 days. Four controls that received no vaccinations, but were infected in the same manner and with the same dosage, lived on an average of 24½ days. The vaccinations and infecting dose were given intravenously.

In neither the calves nor the rabbits did the number of vaccinations seem to have any marked relation to the degree of immunity possessed by the animal.

MISCELLANEOUS EXPERIMENTS.

Several animals were given a large number of vaccinations extending over a long period of time, with several objects in view, among which was to determine the harmlessness of the Vaccine, the immunizing value of their milk and the serum of their blood, as well as the outcome of the living tubercle bacilli constituting the Vaccine. Some portions of this work were never carried to completion, but the treatment and results of each animal are interesting and give sufficient information on certain phases of the problem to warrant recording them in conjunction with this report.

Animal No. 16026. A red and white yearling heifer was given the following vaccinations:

1903.

January 31st, injected in jugular vein, 5 cc. Standard Suspension Culture M.

February 9th, injected in jugular vein, 10 cc. Standard Suspension Culture M.

February 18th, injected in jugular vein, 10 cc. Standard Suspension Culture M.

March 2nd, injected in jugular vein, 10 cc. Standard Suspension Culture M.

March 21st, injected in jugular vein, 12½ cc. Standard Suspension Culture M.

April 4th, injected in jugular vein, 12½ cc. Standard Suspension Culture M.

April 10th, injected in jugular vein, 12½ cc. Standard Suspension Culture M.

April 18th, injected in jugular vein, 15 cc. Standard Suspension Culture M.

April 25th, injected in jugular vein, 15 cc. Standard Suspension Culture M.

May 1st, injected in jugular vein, 15 cc. Standard Suspension Culture M.

May 9th, injected in jugular vein, 18½ cc. Standard Suspension Culture M.

May 16th, injected in jugular vein, 18½ cc. Standard Suspension Culture M.

May 23rd, injected in jugular vein, 18½ cc. Standard Suspension Culture M.

June 1st, injected in jugular vein, 20 cc. Standard Suspension Culture M.

June 12th, injected in jugular vein, 20 cc. Standard Suspension Culture M.

June 18th, injected in jugular vein, 20 cc. Standard Suspension Culture M.

July 15th, injected in jugular vein, 22½ cc. Standard Suspension Culture M.

July 23rd, injected in jugular vein, 22½ cc. Standard Suspension Culture M.

July 28th, injected in jugular vein, 22½ cc. Standard Suspension Culture M.

July 30th, injected in jugular vein, 25 cc. Standard Suspension Culture M.

August 1st, injected in jugular vein, 25 cc. Standard Suspension Culture M.

August 3rd, injected in jugular vein, 25 cc. Standard Suspension Culture M.

August 6th, injected in jugular vein, 27½ cc. Standard Suspension Culture M.

August 10th, injected in jugular vein, 27½ cc. Standard Suspension Culture M.

August 12th, injected in jugular vein, 27½ cc. Standard Suspension Culture M.

August 14th, injected in jugular vein, 30 cc. Standard Suspension Culture M.

August 19th, injected in jugular vein, 30 cc. Standard Suspension Culture M.

August 22nd, injected in jugular vein, 30 cc. Standard Suspension Culture M.

August 25th, injected in jugular vein, 32½ cc. Standard Suspension Culture M.

August 29th, injected in jugular vein, 32½ cc. Standard Suspension Culture M.

August 31st, injected in jugular vein, 32½ cc. Standard Suspension Culture M.

September 2nd, injected in jugular vein, 35 cc. Standard Suspension Culture M.

September 5th, injected in jugular vein, 35 cc. Standard Suspension Culture M.

September 7th, injected in jugular vein, 35 cc. Standard Suspension Culture M.

September 8th, injected in jugular vein, 40 cc. Standard Suspension Culture M.

September 9th, injected in jugular vein, 40 cc. Standard Suspension Culture M.

September 11th, injected in jugular vein, 40 cc. Standard Suspension Culture M.

November 7th, injected in jugular vein, 20 cc. Standard Suspension Culture M.

November 9th, injected in jugular vein, 20 cc. Standard Suspension Culture M.

November 12th, injected in jugular vein, 20 cc. Standard Suspension Culture M.

November 16th, injected in jugular vein, 30 cc. Standard Suspension Culture M.

November 19th, injected in jugular vein, 30 cc. Standard Suspension Culture M.

November 21st, injected in jugular vein, 30 cc. Standard Suspension Culture M.

November 24th, injected in jugular vein, 30 cc. Standard Suspension Culture M.

December 1st, injected in jugular vein, 40 cc. Standard Suspension Culture M.

December 3rd, injected in jugular vein, 40 cc. Standard Suspension Culture M.

December 7th, injected in jugular vein, 40 cc. Standard Suspension Culture M.

December 16th, injected in jugular vein, 40 cc. Standard Suspension Culture M.

December 23rd, injected in jugular vein, 40 cc. Standard Suspension Culture M.

December 28th, injected in jugular vein, 40 cc. Standard Suspension Culture M.

December 31st, injected in jugular vein, 40 cc. Standard Suspension Culture M.

1904.

January 2nd, injected in jugular vein, 40 cc. Standard Suspension Culture M.

January 4th, injected in jugular vein, 40 cc. Standard Suspension Culture M.

January 29th, injected in jugular vein, 40 cc. Standard Suspension Culture M.

January 30th, injected in jugular vein, 40 cc. Standard Suspension Culture M.

February 1st, injected in jugular vein, 40 cc. Standard Suspension Culture M.

February 2nd, injected in jugular vein, 50 cc. Standard Suspension Culture M.

February 11th, injected in jugular vein, 50 cc. Standard Suspension Culture M.

February 15th, injected in jugular vein, 50 cc. Standard Suspension Culture M.

February 21st, injected in jugular vein, 50 cc. Standard Suspension Culture M.

February 23rd, injected in jugular vein, 50 cc. Standard Suspension Culture M.

February 25th, injected in jugular vein, 50 cc. Standard Suspension Culture M.

March 1st, injected in jugular vein, 50 cc. Standard Suspension Culture M.

March 3rd, injected in jugular vein, 50 cc. Standard Suspension Culture M.

March 5th, injected in jugular vein, 50 cc. Standard Suspension Culture M.

March 7th, injected in jugular vein, 50 cc. Standard Suspension Culture M.

March 16th, injected in jugular vein, 60 cc. Standard Suspension Culture M.

March 18th, injected in jugular vein, 60 cc. Standard Suspension Culture M.

March 26th, injected in jugular vein, 60 cc. Standard Suspension Culture M.

March 28th, injected in jugular vein, 60 cc. Standard Suspension Culture M.

March 30th, injected in jugular vein, 60 cc. Standard Suspension Culture M.

April 1st, injected in jugular vein, 60 cc. Standard Suspension Culture M.

April 5th, injected in jugular vein, 60 cc. Standard Suspension Culture M.

April 8th, injected in jugular vein, 60 cc. Standard Suspension Culture M.

April 11th, injected in jugular vein, 60 cc. Standard Suspension Culture M.

April 18th, injected in jugular vein, 60 cc. Standard Suspension Culture M.

April 20th, injected in jugular vein, 60 cc. Standard Suspension Culture M.

April 22nd, injected in jugular vein, 60 cc. Standard Suspension Culture M.

April 25th, injected in jugular vein, 60 cc. Standard Suspension Culture M.

April 29th, injected in jugular vein, 60 cc. Standard Suspension Culture M.

May 24th, injected in jugular vein, 60 cc. Standard Suspension Culture M.

May 27th, injected in jugular vein, 60 cc. Standard Suspension Culture M.

May 31st, injected in jugular vein, 60 cc. Standard Suspension Culture M.

June 2nd, injected in jugular vein, 60 cc. Standard Suspension Culture M.

June 6th, injected in jugular vein, 60 cc. Standard Suspension Culture M.

June 9th, injected in jugular vein, 60 cc. Standard Suspension Culture M.

June 11th, injected in jugular vein, 60 cc. Standard Suspension Culture M.

June 13th, injected in jugular vein, 60 cc. Standard Suspension Culture M.

June 17th, injected in jugular vein, 60 cc. Standard Suspension Culture M.

June 20th, injected in jugular vein, 60 cc. Standard Suspension Culture M.

June 22nd, injected in jugular vein, 60 cc. Standard Suspension Culture M.

June 28th, injected in jugular vein, 60 cc. Standard Suspension Culture M.

June 30th, injected in jugular vein, 60 cc. Standard Suspension Culture M.

July 2nd, injected in jugular vein, 60 cc. Standard Suspension Culture M.

July 6th, injected in jugular vein, 60 cc. Standard Suspension Culture M.

July 8th, injected in jugular vein, 60 cc. Standard Suspension Culture M.

July 11th, injected in jugular vein, 60 cc. Standard Suspension Culture M.

July 13th, injected in jugular vein, 60 cc. Standard Suspension Culture M.

July 15th, injected in jugular vein, 60 cc. Standard Suspension Culture M.

July 18th, injected in jugular vein, 60 cc. Standard Suspension Culture M.

July 20th, injected in jugular vein, 60 cc. Standard Suspension Culture M.

July 22nd, injected in jugular vein, 60 cc. Standard Suspension Culture M.

July 25th, injected in jugular vein, 60 cc. Standard Suspension Culture M.

August 10th, injected in jugular vein, 60 cc. Standard Suspension Culture M.

August 12th, injected in jugular vein, 60 cc. Standard Suspension Culture M.

August 15th, injected in jugular vein, 60 cc. Standard Suspension Culture M.

August 17th, injected in jugular vein, 60 cc. Standard Suspension Culture M.

August 19th, injected in jugular vein, 60 cc. Standard Suspension Culture M.

December 16th, injected in jugular vein, 20 cc. Standard Suspension Culture M.

December 21st, injected in jugular vein, 20 cc. Standard Suspension Culture M.

December 24th, injected in jugular vein, 20 cc. Standard Suspension Culture M.

December 27th, injected in jugular vein, 40 cc. Standard Suspension Culture M.

December 29th, injected in jugular vein, 40 cc. Standard Suspension Culture M.

1905.

January 4th, injected in jugular vein, 40 cc. Standard Suspension Culture M.

January 7th, injected in jugular vein, 40 cc. Standard Suspension Culture M.

January 13th, injected in jugular vein, 40 cc. Standard Suspension Culture M.

January 26th, injected in jugular vein, 40 cc. Standard Suspension Culture M.

February 4th, injected in jugular vein, 40 cc. Standard Suspension Culture M.

February 14th, injected in jugular vein, 40 cc. Standard Suspension Culture M.

March 16th, injected in jugular vein, 40 cc. Standard Suspension Culture M.

May 13th, injected in jugular vein, 20 cc. Standard Suspension Culture M.

May 31st, injected in jugular vein, 20 cc. Standard Suspension Culture M.

June 6th, injected in jugular vein, 20 cc. Standard Suspension Culture M.

August 7th, injected in jugular vein, 20 cc. Standard Suspension Culture M.

1906.

January 24th, injected in jugular vein, 2½ cc. Standard Suspension Culture M.

January 31st, injected in jugular vein, 5 cc. Standard Suspension Culture M.

February 7th, injected in jugular vein, 7½ cc. Standard Suspension Culture M.

February 14th, injected in jugular vein, 10 cc. Standard Suspension Culture M.

February 21st, injected in jugular vein, 12½ cc. Standard Suspension Culture M.

March 1st, injected in jugular vein, 15 cc. Standard Suspension Culture M.

March 20th, injected in jugular vein, 17½ cc. Standard Suspension Culture M.

This animal received in all 131 vaccinations from January 31, 1903, to March 20, 1905, amounting to 5,120½ cc. of Vaccine which represented 6.6 grams (6,600 milligrams) of dried tubercle bacilli. During a greater part of the time this animal was exposed to infection by being kept in an infected stable and frequently came in contact with tuberculous animals. She remained in good flesh and gradually gained in weight. Two tuberculin tests were applied with a double dose of tuberculin. One on December 5, 1904, with a positive result and the other on November 3, 1905, with a negative result. Daily temperature records were kept and on some occasions, though with no definite regularity, she would give a reaction following the injection of the Vaccine. The animal gave birth to a calf sometime during the Spring of 1906 and died rather suddenly on April 15, 1906, and the autopsy is given in full as recorded by the late Dr. Leonard Pearson.

Post-mortem animal 16026. Died April 15, 1906.

This animal died at 5.30 P. M. on the 15th, and the post-mortem was made at 5 A. M. on the morning of the 16th by Drs. Pearson and Deubler. The carcass was slightly bloated by the accumulation of gas in the stomachs. There was some odor of putrefaction. The animal was well nourished and showed considerable subcutaneous and perivascular fat.

AUTOPSY.

"The heart contains large clots of blood, though on section is found to be normal throughout.

"The right lung is uniform in texture, and contains much blood which may be washed out, leaving spongy lung tissue. The left lung is spongy, uniform in texture, and contains less blood than the right lung. There is no evidence of tuberculosis, or any other pulmonary disease.

"Both of the Bronchial, and all of the Mediastinal Lymphatic Glands are red and softened from post-mortem alteration. They are not enlarged and show no nodules or evidence of tuberculosis. The Post-pharyngeal Lymphatic Glands are normal. The liver is yellowish brown, and at the point where it is in contact with the stomach, is stained greenish in color. It is somewhat friable, though all the changes noted appear to be post-mortem. No nodules, abscesses, or evidence of tuberculosis found in any part of the organ. The portal lymphatic gland is normal.

"The right kidney is friable, yellowish red in color, and its surface presents a finely mottled condition—red points being surrounded by yellowish brown color. The left kidney is in a similar condition. The spleen is normal.

"The stomachs and intestines are somewhat discolored, having a greenish hue, which is no doubt, post-mortem. The Mesenteric Lymphatic glands are not enlarged, and show no nodules.

"The udder is rather large, vascular, and contains milk. The Supramammary Lymphatic Glands are normal.

"The bladder is empty except for a small amount of thin, yellowish, muddy fluid, which microscopically consisted of pavement and spindle epithelial cells—no columnar cells or casts. The right horn of the uterus wall is somewhat thickened. The horn contains a small quantity of turbid, reddish or violet colored fluid in which small blood clots float, shreds of mucus, patches of mucous membrane from size of a grain of wheat to size of a half-dollar. In this fluid there is found one placental cotyledon. The mucous membrane of this horn is greenish blue and eroded. The left horn of the uterus is normal.

"The brain was removed by bisecting the head longitudinally. The membranes covering the brain are clear. There is, however, a deposit of a thin layer of yellowish gray material of a consistency of fibrin on each side of the middle of the cerebrum and extending to the inferior surface and below backward to the medulla This deposit is thickest in the fissures, where it is 1-12″ thick, gradually thinning over the convolutions until its disappearance. In this deposit there are a few yellowish points 1-32″ to 1-20″ in diameter."

Staining smears from this deposit in the fissures, tubercle bacilli could be readily found. Each was long and thin. Cultures made from the fissures, revealed a staphylococcus. The brain had not been handled or removed in an aseptic manner.

Dr. D. J. McCarthy on gross examination of the brain, gives it as his opinion, that the condition was one of tubercular meningitis associated with a mixed infection.

HISTOLOGICAL EXAMINATION.

"Lung, macroscopically, shows no evidence of tuberculosis or other pulmonary disease. Microscopically, moderate amount of congestion and in areas marked oedema with collopse are seen. One small nodule in the lung, shows early abscess formation, in the centre of which tubercle bacilli are found.

"In another section, a desquamated bronchitis is seen with possibly an early tubercle.

"Liver, macroscopically, all changes appear to be post-mortem, no nodular abscess or evidence of tuberculosis.

"Microscopically, liver shows slight increase in interlobular connective tissue, capsule moderately thickened. In some sections the blood vessels are filled with granular detritus, probably bacteria, post-mortem change.

"Kidney, grossly appeared flaccid yellowish red. Surface freely mottled, red parts being surrounded by yellowish brown color. Microscopically, a slight cloudy swelling is all that could be detected.

"Udder, grossly was rather large, contained milk and rather vascular. Microscopically, found to be slightly congested, otherwise normal.

"Uterus, section taken from right horn the walls of which are somewhat thickened. Horn contained a turbid reddish or violet colored fluid in which small blood clots float shreds of mucous patches and mucous membrane.

"Microscopically, it was found, that the endormetrium showed a slight exudate, the basement membrane of which is distinctly congested with considerable hyalin change around the vessels. In the deeper structure there are plugs of rounded bodies, staining deeply with haemotoxylin, having a ray like structure, the periphery is denser than the deeper structure. No tubercle bacilli found in the specimen stained.

"Brain, grossly there appears to be a thickening or rather a deposit which favors the fissures of the brain, where it is 1-12 inch thick, gradually thinning over the involutions until invisible. Tubercle bacilli were found in the scrapings made from the deposit in one of the fissures. Microscopically a typical tubercular meningitis with caseation and giant cells is seen."

Two guinea pigs inoculated with emulsion of udder tissue, one died of peritonitis and the other one was killed three months later and found to be normal. Guinea pigs inoculated with an emulsion of kidney remained normal. Guinea pigs and rabbits inoculated with emulsion of brain died of peritonitis. The organism causing the same was not isolated and studied.

The work on this animal shows that Culture M was not virulent for grown animals or else the early injections of Vaccine gave protection for the enormous quantities given later. This animal evidently died of a tuberculous meningitis aggravated by a mixed infection. This corresponds to the results of McFadyean as reviewed in Part II of this paper.

A number of rabbits and guinea pigs were treated with the serum of the blood of the above animal and afterwards infected with virulent bovine tubercle bacilli. It was not found that there was any appreciable difference in the length of the life of the animals treated with the serum than the controls inoculated in a similar manner with bovine tubercle bacilli.

It was found that the serum from the blood of this animal had a greater inhibitory effect upon the growth of tubercle bacilli on glycerine bouillon than serum from a normal animal. Flasks to which was added approximately 10 per cent. of serum contained very little growth after one month in the incubator, while flasks containing the same amount of serum from a normal cow were three-fourth covered with growth. Control flasks were fully grown in this time.

Animal No. 45200, a bay gelding, was given the following intravenous vaccinations:

1902.

December 11th, injected in jugular vein, 10 cc. Standard Suspension Culture M.

1905.

January 2nd, injected in jugular vein, 15 cc. Standard Suspension Culture M.

August 6th, injected in jugular vein, 1 cc. Standard Suspension Culture M.

August 12th, injected in jugular vein, 2 cc. Standard Suspension Culture M.

August 19th, injected in jugular vein, 3 cc. Standard Suspension Culture M.

On August 29th, ten days following the last vaccination, this animal was killed in a very bad condition and found to have extensive miliary tuberculosis of the lungs. This would indicate that Culture M was much more virulent for horses than for cattle.

Two cows, No. 143 and No. 144, were given a number of vaccinations with the object of ascertaining whether their offspring had any greater resistance to tuberculosis than an ordinary calf, and further to determine whether their milk had any immunizing property. These two cows were purchased on the open market and tested with tuberculin with a negative result. They were both given the following vaccinations.

1904.

December 21st, injected in jugular vein, $2\frac{1}{2}$ cc. Standard Suspension Culture M.

December 29th, injected in jugular vein, 5 cc. Standard Suspension Culture M.

1905.

January 13th, injected in jugular vein, 7½ cc. Standard Suspension Culture M.

February 3rd, injected in jugular vein, 10 cc. Standard Suspension Culture M.

February 14th, injected in jugular vein, 12½ cc. Standard Suspension Culture M.

May 13th, injected in jugular vein, 5 cc. Standard Suspension Culture M.

June 6th, injected in jugular vein, 10 cc. Standard Suspension Culture M.

August 7th, injected in jugular vein, 12½ cc. Standard Suspension Culture M.

October 5th, injected in jugular vein, 15 cc. Standard Suspension Culture M.

1906.

January 24th, injected in jugular vein, 2½ cc. Standard Suspension Culture M.

January 31st, injected in jugular vein, 5 cc. Standard Suspension Culture M.

February 7th, injected in jugular vein, 7½ cc. Standard Suspension Culture M.

February 14th, injected in jugular vein, 10 cc. Standard Suspension Culture M.

February 21st, injected in jugular vein, 12½ cc. Standard Suspension Culture M.

March 1st, injected in jugular vein, 15 cc. Standard Suspension Culture M.

March 20th, injected in jugular vein, 17½ cc. Standard Suspension Culture M.

April 5th, injected in jugular vein, 20 cc. Standard Suspension Culture M.

April 25th, injected in jugular vein, 22½ cc. Standard Suspension Culture M.

May 10th, injected in jugular vein, 25 cc. Standard Suspension Culture M.

In all they received 19 vaccinations consisting of 217½ cc. of Vaccine, representing 0.28 gram (280 milligrams) dried tubercle bacilli. Both animals were tuberculin tested on Nov. 3rd. 1905 and No. 143 gave a positive reaction, while No. 144 gave a questionable reaction. They were both tuberculin tested again on February 11, 1907, and on April 25, 1907, and neither of them gave a distinct reaction. Tuberculin of three times the regular strength was used for the test of November 3, 1905, while regular strength tuberculin was used for the two tests in 1907. These cows were at all times exposed to a moderate degree of infection to tuberculosis by being kept in infected stables and in more or less direct contact with tuberculous animals.

AUTOPSIES.

Animal No. 144 in February 1908 developed a large knee joint which was painful and stiff. On March 23rd, 1908, this animal died after a severe attack of diarrhoea, the cause of which was not known. The general condition of the animal was good. Lungs and Bronchial Glands normal. Posterior Mediastinal Gland contained an abscess cavity filled with yellow caseous material. Portal Lymphatic Gland and a number of Mesenteric Lymphatic Glands were enlarged and on section a yellow creamy pus escaped. The Post-pharyngeal and Inguinal Lymphatic Glands were enlarged and caseous. Brain and Meninges normal. Guinea pigs and rabbits inoculated with caseous material found in the Mediastinal, Mesenteric and Post-pharyngeal Lymphatic Glands developed tuberculosis. Guinea pigs inoculated from the spinal fluid remained normal. The exact cause of the diarrhoea could not be determined from the autopsy.

Animal No. 143. This animal was exposed to more or less infection to tuberculosis until killed January 21, 1913. This animal showed a chronic tuberculosis of the pleura covering the lungs and diaphragm, the lungs, the pericardium, the mediastinal lymphatic glands, liver, portal lymphatic gland, syleen, omentum, mesenteric lymphatic glands and peritoneum.

On May 25, 1906, two weeks following the last vaccination, cow No. 143 dropped a calf. This calf was allowed to run and nurse from both these cows No. 143 and No. 144 until it was about eight months old. It was afterwards exposed to a more severe tuberculous infection and was killed December 6, 1907, at the age of nineteen months. At autopsy one bronchial lymphatic gland contained a caseous nodule $\frac{1}{2}$ x $\frac{3}{4}$ of an inch. The only other lesions were found in several mesenteric glands which contained dry caseous nodules well encapsulated. It appears that this calf had more than a normal resistance to tuberculosis. Other control calves exposed to the same degree of infection developed tuberculosis to a much more marked degree. Can it be attributed to the milk of these cows or to its mother's blood during the period of gestation, at which time she was receiving injections of Vaccine? A second calf was born to this cow the following year which did not show the same degree of resistance.

On June 5, 1906, approximately three weeks following the last vaccination, cow No. 144 gave birth to a calf which died 11 days later on June 16th. On autopsy the lungs were found attached to the chest wall in a number of places. The liver was attached to the diaphragm and spleen in a similar manner. Smears made from the liver revealed an organism which was believed to be the tubercle bacillus. Guinea pigs inoculated with an emulsion of the liver developed tuberculosis.

The animals in the foregoing experiments were tuberculin tested at various intervals during the progress of the work. It was found that the vaccinated animals would respond to tuberculin for an indefinite time following the vaccinations. On autopsy a number of these animals were entirely free from any macroscopic lesions of tuberculosis. In some instances animals would give a positive reaction and in a few months a negative reaction, and then after another interval of a few months another positive reaction. This, however, was not found to be true in the majority of cases.

In analyzing the tuberculin records of a number of herds in which the calves had been vaccinated when three to four months of age and then tuberculin tested with the herd, it was found that some of the animals lost their sensitiveness to tuberculin as early as four months following the vaccinations, while again other animals in the same herd and vaccinated in a similar manner retained their sensitiveness to tuberculin for twenty months. These animals were in non-infected herds or in herds in which not more than 2% to 3% of the animals were afflicted with tuberculosis and these only in the early stages as it was the policy of the owner to remove all reacting cattle as soon as discovered. Of these animals which came to autopsy none of them contained macroscopic lesions of the disease.

It was noticed in some of these experiments and especially in some herds in which vaccination was used as a means of controlling the disease that a few of the vaccinated animals would develop a large joint or a small abscess in the udder, the carpus or knee joint being the one most usually affected. The joint would enlarge and become extremely painful. The soreness would gradually disappear but the enlargement with induration of the surrounding tissues would remain. On autopsy these joints were found to contain an excess of synovial fluid but rarely any erosion of the cartilagenous surface. The capsule of the joint was greatly thickened. On several occasions tubercle bacilli were found in the synovial fluid. In some of the young heifers there was an enlargement of one or more quarters of the udder. Hard masses the size of a walnut could be felt in the udder tissue. In some instances they would disappear entirely while again they would remain for a year or more. At the time of autopsy these nodules or hard masses were found to contain tubercle bacilli. It seems reasonable to believe that these knee and udder conditions were due to clumps of tubercle bacilli in the vaccine. Such animals generally were free from tuberculosis in other parts of the body. Is it not possible that these bacilli remaining alive in the joint or udder was responsible for the continued immunity shown by these animals.

I am of the opinion that the tubercle bacilli constituting the vaccine circulate in the blood or remain in the glands for many months following the vaccinations. Further, I believe that it is possible for them to remain alive in the glands without producing any pathological changes in the gland tissue. It has been suggested that we will eventually obtain the highest and most lasting immunity by using a vaccine consisting of tubercle bacilli that are slowly absorbed and at the same time are not of sufficient virulence to infect the animal. Before better results may be expected in the production of immunity against tuberculosis more knowledge is required of the relation of the tubercle bacilli in the vaccine to the degree and length of immunity conferred upon the animal. Is the immunity lost as soon as the tubercle bacilli are disposed of or destroyed by the natural defenses of the body?

In making a careful analysis of this work I have come to the following conclusions:

I. Intravenous injections of tubercle bacilli from human sources non-virulent for cattle, are capable of conferring an immunity in cattle against tuberculosis sufficient to withstand natural infection by association with tuberculous cows.

II. The length of the immunity conferred has not been determined definitely, but it is believed to gradually diminish after two and one-half years.

III. The vaccinated animal during the period of vaccination and for some weeks afterwards is more liable to contract tuberculosis than a normal animal. The natural resistance of the animal is apparently lowered during the time of vaccination.

IV. The interval between the vaccinations should be of a sufficient length to allow any reaction following the previous vaccination to entirely subside.

V. The degree of immunity obtained in the animal depends to a certain extent upon the number of vaccinations and the amount of Vaccine administered.

VI. The Vaccine should be prepared so it contains no clumps of bacilli and should be administered fresh.

VII. A number of the vaccinated animals may give a typical tuberculin reaction following the vaccinations for a period of twenty months. These animals may or may not show lesions of tuberculosis at autopsy.

VIII. Vaccine administered to animals already infected with tuberculosis is capable of retarding or holding in check the progress of the disease.

IX. The milk from immunized cows when fed over a long period of time appears to increase the resistance of calves and pigs.

X. Vaccination of calves against tuberculosis is of assistance in the eradication of tuberculosis from a herd if done under the proper conditions.

XI. Until further knowledge is obtained in regard to the destruction or outcome of the living tubercle bacilli constituting the Vaccine, no practical method for the immunization of milk-producing animals under ordinary conditions can be advocated.

PART II.

A Review of the Production of Immunity in Cattle against Tuberculosis and the Use of Tuberculin.

A REVIEW OF THE PRODUCTION OF IMMUNITY IN CATTLE AGAINST TUBERCULOSIS AND THE USE OF TUBERCULIN.

A. The Protective Vaccination of Cattle against Tuberculosis.

Efforts toward the production of immunity in animals against tuberculosis may be said to have commenced with the discovery of tuberculin by Koch in 1890. Numerous experiments with tuberculin as a preventive and as a cure for tuberculosis were made for a number of years following the discovery of this substance. It was found that while tuberculin has its specific effect upon the lesions of tuberculosis and in some instances causes the lesions to become encapsulated, to recede or to disappear, this effect is by no means constant and is not sufficiently frequent to make this mode of treatment of any practical value in the immunization or treatment of animals against the disease. It appears that the effect of tuberculin was to produce a toxic rather than a bacterial immunity. After it was found that satisfactory results could not be obtained with tuberculin and watery glycerine extracts of tubercle bacilli, attempts were made to attain better success with toxins which were prepared from tubercle bacilli in different ways, and reports of such experiments have been published by Koch, Klebs, von Ruck, von Behring, Hirschfelder and Maragliano, but these trials were unsatisfactory.

In 1897 Koch came to the conclusion that it is necessary in the treatment against Tuberculosis to produce an immunity, not only against the toxins of the tubercle bacilli, but it is also necessary to produce a bacterial immunity. He therefore inoculated the animals on which he experimented with tubercle bacilli, both killed as well as living. The results he obtained, however, were not encouraging, as the bacilli were not sufficiently absorbed or the animals were made ill and death even followed the injections.

In 1899 Dixon published an article upon "The possibility of establishing tolerance for the tubercle bacillus" in which he claimed that avirulent and atypical tubercle bacilli when taken into the body disappeared and gave rise to a more or less resistance to tuberculous infection. In 1891 the same author published an article entitled "Fluid of Dixon; the possibility of checking the tubercular process in man by the agency of a metabolic product of the tubercle bacillus," stating that extracts from the tuberculous bacillary growths injected into the animal economy would prevent the growth of the tubercle bacilli that inhabit the tissues. He also published some experiments upon animals with his watery extract of tubercle bacilli.

In 1892 and 1893 Trudeau found that by subcutaneous inoculations of living cultures of avian tubercle bacilli, he was able to increase the resistance of a rabbit to infection with virulent mammalian cultures. He also succeeded in immunizing rabbits to such an extent that when inoculated in the eye with mammalian cultures the first inflammatory reaction gradually disappeared, leaving the eye in normal condition, while similar inoculations in control animals led to the destruction of the eye.

In 1894 De Schweinitz of the U. S. Bureau of Animal Industry reported in the Medical News some experiments made upon guinea pigs in which these animals were inoculated with tubercle bacilli of the human type cultivated upon glycerin beef broth for about twenty generations. These animals with controls were afterwards injected with tuberculous material from a cow. The treated guinea pigs remained free from tuberculosis while the check animals inoculated with the same tuberculous material from the cow died of tuberculosis within seven weeks. De Schweinitz further reported injecting into cattle large quantities of human tubercle bacilli, the injections being made subcutaneously, intravenously and into the peritoneal cavity. He established the fact that on gradually increasing the amounts injected the animals were able to stand larger amounts of tubercle bacilli without injury.

McFadyean reported in the Journal of Comparative Pathology and Therapeutics for June, 1901, and March, 1902, upon some experiments regarding immunization of cattle against tuberculosis. He started with material that was only slightly virulent and with moderately large doses of tuberculin. He inoculated four cattle intravenously with emulsions of tuberculous material and cultures from various sources. One of these cattle which had responded to the tuberculin test and was no doubt tubercular upon the beginning of the experiment, was given about 150 cc. of tuberculin in divided doses before inoculation. Fifteen weeks after inoculation this animal was killed and was found to contain but one tubercle the size of a pea and completely calcified in a mesenteric gland. Two control cattle inoculated with an equal dose of the same material died of generalized tuberculosis. Of the other cattle of the series, one was tubercular at the beginning of the experiment. All of these were inoculated intravenously from seven to eleven times during a period of from two to three years with emulsions of tuberculous materials and with cultures from various sources. It is interesting to note that the first inoculation upon each of the cows that was free from tuberculosis at the beginning of the experiment, was made with avian material which was probably of very low virulence for cattle. The cattle so inoculated died of tuberculosis after two to three years from the beginning of the experiment, and in each case the chief lesions were in the kidneys and the brain or its covering membranes. The cerebral lesion appears to have been the immediate cause of death in each instance. There can be no doubt that these animals were remarkably resistant to tuberculosis because they lived for months or years after repeated inoculations with large quantities of material of proven virulence for cattle. From the results of his experiments he draws the conclusion that the capacity for resistance required by the animals through the repeated injections was not absolute, but was nevertheless much higher than that attained by any other method of immunization.

Von Behring announced December 22, 1901, that he was engaged in studying the immunization of cattle against tuberculosis and has published a number of reports upon the results of his work. von Behring has made a very large number of experiments in this field. He has endeavored to immunize animals against tuberculosis by the use of tuberculin, other tuberculosis toxins, by antitoxins, by the use of dead tubercle bacilli, tubercle bacilli weakened by chemical agents, or by the use of living cultures of low virulence. Animals treated by von Behring have been tested as to their resistance to tuberculosis by subcutaneous and intravenous inoculations with tuberculous material from cattle and with cultures of virulent tubercle bacilli of the bovine type as well as by associating the treated cattle with animals in an infected herd. Some of the animals immunized by von Behring have been tested as to their resistance to tuberculosis by others. For this purpose cattle have been sent from the Institute in Marburg to Eber in Dresden, Schlegel in Frieburg, Lorenz in Darmstad and to others. Further details of the treatment of the animals under the von Behring method and the result of the test of their immunity will be referred to later.

In the Proceedings of the American Medical Association, 1897, McFarland of Philadelphia reports upon the treatment of an ass with increasing doses of tuberculin up to 150 cc. The serum from the blood of the ass so treated was termed "Anti-tuberculin" and was used in experiments on guinea pigs and therapeutic trials were also made on tuberculous persons. The work on guinea pigs comprised experiments to determine the effect of the serum on healthy animals, on animals already inoculated with tuberculosis and to determine its immunizing value and its ability to neutralize tuberculin and to counteract the effects of tuberculin administered to tuberculous guinea pigs. It was shown that tuberculin was not injurious to healthy guinea pigs, that it would not cure tuberculosis in guinea pigs and that it was not effective as an immunizing agent. It did, however, as shown by one set of experiments, appear to have the effect of counteracting the effect of tuberculin on tuberculous guinea pigs. This result was not clearly shown in a second series of experiments.

Paterson endeavored to immunize rabbits and guinea pigs by injections of dead cultures of avian tubercle bacilli and also by injections of the serum of fowls that had received large doses of dead avian tubercle bacilli. He found that by this means animals could be immunized to inoculation of tuberculosis to a small extent.

Thomassen in 1901 conducted some experiments in which he found that cattle which had received preliminary intravenous injections of human tubercle bacilli were more resistant to infection from bovine tubercle bacilli than animals which had not received this preliminary treatment. One of his experiments went to show that even one single injection with human tubercle bacilli produced a high degree of resistance which, however, was not sufficient to protect cattle against infection.

Neufeld carried on in Koch's Institute in Berlin an extensive series of experiments upon the production of immunity by the use of living tubercle bacilli of low virulence. He ascertained by prolonged experimentation that it was not possible to obtain immunity in experimental animals by the use of toxins or dead tubercle bacilli. However, he found it possible to immunize goats, mules and cattle by the intravenous injection of live human tubercle bacilli against fatal doses of virulent bovine tubercle bacilli.

According to experiments, Koch, Schuetz, Neufeld and Missner reported in 1905, it is possible by a single injection of 10 to 30 milligrams of human tubercle bacilli or with attenuated bacilli of "perlsucht" to immunize cattle against highly virulent bovine bacilli.

The above experiments brought about a large amount of research work upon the immunization of cattle against tuberculosis by experimenters throughout the world. Some of the investigators used Vaccine from fresh cultures of human tubercle bacilli or attenuated cultures of bovine tubercle bacilli, while others used bacilli of fowls as well as bacilli of cold-blooded animals. Various methods of vaccination were tried, including the subcutaneous method as recommended by von Baumgarten, Lignieres and Hutyra. Calmette and Guerin as well as Roux and Vallee tried a method of introducing live as well as dead tubercle bacilli into the digestive tract of calves, with the object of increasing the natural resistance of the calf. The same method was pursued by Arloing in goats. Pearson and Gilliland reported upon the attenuation of tubercle bacilli by placing them for a time in the abdominal cavity of a calf enclosed in a collodion capsule. Their object was to influence the tubercle bacilli in such a manner that it would be better adapted for the production of immunity in cattle. Heymans devised a method of immunization by introducing under the skin of cattle live tubercle bacilli contained in reed bags. Other investigators endeavored to bring about immunity by the use of living non-virulent human tubercle bacilli by introducing them into the subcutaneous tissue, the abdominal cavity and the peritoneal cavity. We shall more fully describe the separate methods and give in brief the results attained by the use of these methods.

VON BEHRING METHOD OF PROTECTIVE VACCINATION.

Following the example of Jenner and Pasteur, it was von Behring's object to produce immunity in calves against tuberculosis with a less virulent variety of the human tubercle bacilli. He recommended after a series of preliminary experiments to use a culture of tubercle bacilli from a case of pulmonary tuberculosis which had been grown on serum for from four to six weeks. In his first publication on his method of protective vaccination against tuberculosis in cattle it is stated that this culture had been previously grown for six and one-half years on glycerin bouillon.

The first directions von Behring published in regard to the manner of vaccinating the calf was as follows: To take 0.001 grams of the culture above referred to and suspend the same in 2 cc. of physiological salt solution, and afterwards inject intravenously into an animal five to seven months old which was found free from tuberculosis by the tuberculin test. The animal so treated is to receive four weeks later a dose twenty-five times as large as the initial dose, i. e., 0.025 grams of the same culture suspended in 10 cc. of physiological salt solution and injected intravenously.

This method of vaccination by von Behring later underwent various modifications based on experiences gathered in the meantime. The chief changes he suggested were in the form and amount of the Vaccine as well as the age of the animals to be vaccinated. He further recommended that the Vaccine of the pure cultures of tubercle bacilli be dried in a vacuum at ordinary room temperature, and then to prepare it for injection the powdered Vaccine must be ground in a mortar and thoroughly shaken up in sterile normal saline solution. For the first vaccination each animal was to receive 0.004 grams of the powdered dried tubercle bacilli, (1 immunity unit). For the second vaccination a dose five times as large, 0.02 grams (5 immunity units) of powdered dried tubercle bacilli was recommended. This second injection was to take place not earlier than twelve weeks following the first vaccination. The ages of the animals to be vaccinated should vary as a rule from three weeks to four months. In von Behring's first instructions for the vaccination of calves he set forth that a positive result of the tuberculin test was no contra-indication to the first vaccination provided the animals were not afflicted with clinically demonstrable lesions. He stated further that a thermal reaction following the first vaccination was to be interpreted in the same way as a tuberculin reaction, and if this reaction is strong and continuous for some time, then a second vaccination must be dispensed with. Healthy animals of the age of three weeks to four months do not have to be tested first with tuberculin, even if they belong to a notoriously tuberculous herd. Occasionally it is permissible to vaccinate even older cattle up to two years of age, but only when the animals are perfectly free from symptoms of the disease and when a preliminary tuberculin test shows absolutely no reaction. In the year 194, von Behring designated the age of the animal most suitable for vaccination to be from three weeks to three months.

In 1905 von Behring issued directions for the protective vaccination of cattle against Tuberculosis in which he stated, as a rule only healthy calves from two to twelve weeks old should be vaccinated. In herds in which epidemic diseases are prevalent, especially Calf Pneumonia and Foot and Mouth disease, the vaccinations should not be commenced until after the epidemic has been stamped out. At this time he gave the Vaccine, consisting of the dried pulverized tubercle bacilli, the name of "Bovo-vaccine," and termed the act of injecting the calf with the dried pulverized tubercle bacilli "Bovo-vaccination." In 1906 he announced that in order to facilitate the pulverizing and suspension of the Bovo-vaccine a certain percentage of table salt was incorporated with the tubercle bacilli at the time of its preparation.

It is claimed by some investigators that after a series of practical experiences that Bovo-vaccination if carried out according to directions, is without much risk or danger to the animal. However, some investigators observed immediately following the vaccination of calves certain amount of difficulty in breathing, diarrhoea, disturbed appetite and coughing spells, as well as a trembling of the body. They report that these symptoms which are as a rule more common and more marked at the second vaccination, disappear mostly on the day following the same. In order to avoid as much as possible these symptoms, it is advisable to vaccinate only healthy calves and ones that are not afflicted with pneumonia, and further, it is important to bring the temperature of the Vaccine before injecting to the same temperature as the body. Several authors have reported injurious results following Bovo-vaccination as outlined by von Behring. In one herd on which Marks performed the Bovo-vaccinations five calves out of twenty-six had to be slaughtered several hours after the injections. Casper reports that two calves died a short time after injection without his being able to determine the immediate cause of death. In the case of three other calves tuberculosis developed some weeks following the Bovo-vaccination, which in the opinion of the veterinarians in attendance at the time of the death of the animals and at autopsy could only be traced to the vaccinations. Kern made similar observations.

The various investigators (Rossignol and Vallee, Thommassen, Weber & Titze, Regner & Strenstroem, Nowak, Dammann) called attention to the difference in the virulence of the various preparations of Bovo-vaccine, some being avirulent while others were found to be distinctly virulent for guinea pigs.

According to von Behring, the efficacy of his method of protective Bovo-vaccination is sufficiently proven by the fact "that cattle so treated not only escape with their lives after infection with doses of bovine tubercle bacilli which are fatal to control animals, but they do not show clinically recognizable symptoms of the disease, even after observations extending over months."

The vast importance of this question of immunization of cattle against tuberculosis, both from a public health standpoint as well as from the economical standpoint of the livestock industry, caused a number of investigators in various countries to become active in the verification of von Behring's method of Bovo-vaccination and to devise other methods with the view of obtaining better results. A large number of experiments along this line have been reported showing the results obtained by other investigators.

Ebeling reports upon the vaccination from February, 1903, until the end of 1904 a total of 1,126 calves according to von Behring's method. Of these 1,126 calves 729 had been subjected to a second vaccination at the time his report was issued. For different reasons and various causes 37 of these calves were slaughtered and only one of them was found to be tuberculous, and in the opinion of Ebeling this animal was affected with tuberculosis prior to the time of vaccination.

Roemer states that tuberculin tests have been made on cattle from two to three years old which have been vaccinated when calves, with the result that in about 28% of cases a tuberculous reaction was obtained, while before the introduction of Bovo-vaccination from 80 to 100% of positive reactions would result.

von Behring purchased in 1904 fifty of the Mecklenburg cattle which had received protective bovo-vaccination. These animals, according to Roemer, came from a highly infected herd, and they had been given when they were calves a single protective injection. In 1905 they were subjected to a second vaccination and eight months later to the tuberculin test, at which time three gave a typical reaction and two a doubtful reaction. Hutyra made a number of experiments with calves in which he reported that they showed a marked increase in resistance against tuberculosis after they had been bovo-vaccinated. The method used by him for determining their resistance was infection with virulent bovine tubercle bacilli, either subcutaneously or intravenously. He further stated that it became apparent by continuance of his observations that the resistance which without a doubt increased shortly following the vaccination, later gradually diminished again and after one and one-half years had disappeared completely.

The verification tests of bovo-vaccination made by Thommassen in Holland did not produce favorable results, as reported at the International Veterinary Congress in Budapest in 1905.

Lorenz has made a partial report on the bovo-vaccination according to von Behring's method on 940 animals belonging to 246 herds. Most of the animals were first vaccinated when less than four months old. At the time the report was made the experiment was not complete. There had only been 21 animals of the total number vaccinated slaughtered, and of these 17 were free from tuberculosis, while four showed lesions of the disease. He states that out of the animals vaccinated 78 were subjected to the tuberculin test and 65.4% gave no reaction, while 14.1% gave a slight reaction; 14.1% gave a somewhat stronger reaction, 5.1% gave a strong reaction and 1.3% gave a very marked reaction. He draws attention to the fact that out of the 78 animals tested there were 35% gave a more or less typical reaction. The author of the report was inclined to believe this reaction to tuberculin was due to an over-sensitiveness of the animal, at least as far as some of the animals are concerned. I have been unable to find any further published report upon this experiment.

In France the subject of von Behring's method of bovo-vaccination has been reported upon by Rossignol & Vallee. Their experiments were conducted at Melun and Alfort. They used twenty animals of the age from 6 to 12 months for immunization purposes, and the same number of animals of the same age for control purposes. In their first report, which was based upon the results obtained by testing the resistance or immunity of the vaccinated animal by subcutaneous or intravenous injections of bovine tubercle bacilli. They came to the conclusion that von Behring's method is harmless for the animal to be vaccinated, and that the vaccinated animal can withstand at least for a few months even a natural exposure to infection, and that bovo-vaccination may be capable of protecting the animal against even severe artificial infection. In a second report these authors made public the end results of their experiment including cohabitation tests carried on with two cattle that had been bovo-vaccinated. They also reported upon two animals which were infected one year after the second protective bovo-vaccination, by an intravenous injection of 2 milligrams of a culture of bovine tubercle bacilli. One of the two animals infected in this manner died fifty days later of a very acute extensive pulmonary tuberculosis. The final report of these authors on bovo-vaccination is less favorable than their first report and is as follows:—(1) The marked immunity present about three months after bovo-vaccination against artificial intravenous infection disappears very soon and in some cases has disappeared altogether after one year. (2) The resistance of bovo-vaccinated animals to natural tuberculous infection is not very marked and does not last longer than a few months.

Degive, Stubbe, Mullie and Lienaux have all reported upon experiments upon the protective vaccination of calves against tuberculosis after the von Behring method, as well as the subcutaneous method recommended by von Baumgarten. The testing of the cattle in their experiments as to the resistance or immunity was made by injections of tubercle bacilli, either subcutaneously, intravenously or by feeding, as well as by exposing the vaccinated animal to natural infection. The protective results obtained by them were by no means uniform in all cases. Some of the vaccinated animals appeared to be immune to the test of infection, while others became distinctly ill. In the opinion of the investigators, this difference in results was due apparently to the fact that the Vaccine was not absolutely perfect and that the dosage was not of the same amount for each animal. They drew attention to the fact that the immunity was not attained by the vaccinated animal immediately following the injection of the vaccine, and that during the time which elapses until immunity sets in, there is a possibility of natural infection, and in order to prevent this they recommend that until the definite onset of immunity the animal should be kept in unobjectionable stables. They are of the opinion, according to their report, that the subcutaneous protective vaccination after von Baumgarten method is of equal value to von Behring's method of bovo-vaccination.

In Italy experiments were conducted under the direction of Mazzini by Belfanti & Strazzi, which went toward proving the harmlessness of von Behring's procedure and also to prove the fact that young cattle vaccinated after the method of von Behring, showed increased resistance to subcutaneous infection with active tuberculous material. These authors also point out the fact that the vaccinated animals are highly susceptible to natural infection during a certain length of time following the vaccinations, and they recommend the use of protective measures against infection during this period.

Strelinger reports favorable results from the practical use of the von Behring method. During the years 1902 to 1905 inclusive, he subjected 880 calves to this method. In 1905 he tuberculin-tested 590 of these animals and found that only nine or 1.5% gave a positive reaction. In the case of four of these animals which gave a reaction, it had not been a year since the last protective vaccination, and therefore he considers that in these cases an over-sensitiveness to the tuberculin was present. Against Strelinger's conclusions Hutyra brings forth the objection that his interpretations of the results of the tuberculin test were not strict enough, since he considers as positive only such reactions as designated by von Behring as "long protracted fever reactions with constitutional symptoms." In 1908 Strelinger made an additional report concerning his experience with bovo-vaccination. During the latter part of 1907 and the early part of the year 1908 he tested with tuberculin a total of 686 animals, the oldest of which had been immunized 5½ years and the youngest 2 years. The results of his tests showed that 66 animals or 9.6% gave a positive tuberculin reaction. Strelinger feels from the result of his experience very enthusiastic over bovo-vaccination, for the reason before bovo-vaccination was introduced, two year old animals even when raised in the most careful manner would give positive reactions to the tuberculin test in 50% of cases.

Kern has made several experiments upon the immunization of calves and claims it is possible to immunize animals for a period of two years and over against natural tuberculous infection. Dammann tested the immunity of three calves and a ram which had been bovo-vaccinated after the von Behring method by inoculating them intravenously with virulent bovine tubercle bacilli cultures. Only one of the four animals, a calf, resisted this artificial infection, while the other three animals became tuberculous as well as a number of control animals which had been inoculated in the same manner and with the same dosage and culture. In a second report Dammann publishes the results of some experiments which were conducted by him with the object of giving further information upon the immunity obtained by bovo-vaccination, especially as to the fact whether such immunity could withstand natural infection. To obtain further knowledge on this subject he introduced bovo-vaccinated animals into two herds which contained many animals afflicted with tuberculosis. In both herds the conditions were favorable for the development and spreading of the disease. The animals he selected for immunization were divided into two groups. The first group came from a herd well known to be free from tuberculosis and all of the animals of this group had given a negative reaction to the tuberculin test. The animals of the second group were bought in an apparently healthy condition and in accordance with the proposition of von Behring were not subjected to the tuberculin test. In the first infected herd there were placed four immunized calves from the first group with two controls as well as two immunized calves of the second group and one control. In the second infected herd there were placed two immunized calves from group 1 and two immunized calves from group 2, with two controls. The immunized calves as well as the controls were used in these infected herds for milk production as well as for breeding purposes and were annually subjected to the tuberculin test. The autopsy reports which up to 1910 were collected for all of these experimental animals, showed the following results concerning the immunizing value of bovo-vaccine. Of a total of 11 animals which were subjected to the bovo-vaccinations, 7 animals or 63.6% became infected through cohabitation with tuberculous cattle. Of the five control animals used in the experiments four became affected in the same manner. From these results Dammann comes to the conclusions that von Behring bovo-vaccination alone and by itself is not able to confer on calves a safe protection either against artificial or against natural tuberculous infection.

According to Lelland, who subjected 10 animals that had been bovo-vaccinated to an artificial infection of virulent tuberculous cultures, either intravenously or by other means, came to the conclusion that artificial immunization persists for four months to two years' time.

In the Imperial Health Department Weber & Titze made investigations concerning bovo-vaccination. They used for their experiments a total of twelve calves ranging in age from six to eight weeks. These animals which were vaccinated exavtly according to von Behring's directions, were later tested in regard to their resistance against infection with bovine tubercle baciili. The test was made from two and a half to nine months following this second protective vaccination. This infection was done by injection of the tubercle bacilli either into the blood vessels, subcutaneously, or by inoculation, or by means of food. A group of immunized calves were also exposed to natural infection by association with tuberculous cattle. They report that their results were not satisfactory and the animals proved to be insufficiently protected against artificial as well as against natural infection.

Weber, Titze and Joern made further experiments in their practice with bovo-vaccination. On two estates in Mecklenburg a total of 236 healthy animals were vaccinated with bovo-vaccine. The value of the Vaccine was estimated by means of tuberculin tests one year following the second vaccination and each year thereafter. Judging by the results obtained it seemed that bovo-vaccination conferred a certain amount of immunity on a number of animals temporarily, perhaps for the duration of one year, but on the whole a manifest benefit was not apparent.

Eber has made a rather extensive study of von Behring's method of immunizing calves with bovo-vaccine. He first immunized four calves and kept three animals as controls. He brought them into as intimate contact as possible with animals that had been artificially infected with tuberculosis so as to increase the chances of natural tuberculous infection. After slaughtering the immunized animals as well as the control animals, all of them were found to contain lesions of tuberculosis. A marked difference in the resistance of the immunized animals as compared with that of the control animals against natural infection, could not be established. In addition Eber made extensive tests of bovo-vaccination in his practice on eight estates. In all he immunized 213 cattle. Ten of these animals died before the second vaccination was performed. To decide the value of the vaccination he used mainly tuberculin tests. Out of 148 animals which had been vaccinated strictly in accordance with directions with bovo-vaccine, 119 of these animals gave a positive reaction. He found in his tuberculin tests that the percentage of animals giving a positive reaction was higher the longer the interval between vaccination and the tuberculin test. He therefore comes to the conclusion that bovo-vaccination has produced no appreciable influence on the increasing infection of the younger animals with tuberculosis.

Roemer found that tuberculin tests made on bovo-vaccinated animals on the estates of Archduke Frederick of Austria gave favorable results. These tuberculin tests were made three months after the second protective vaccination. On one of these estates out of thirty vaccinated animals four gave a positive reaction. One of the animals that reacted was killed and was found to be free from tuberculosis. On another estate, out of thirty vaccinated animals two gave a positive reaction. On a third estate, out of thirty-nine vaccinated animals one gave a positive reaction. On a fourth estate out of fifty-five vaccinated calves, four gave a positive reaction. On a fifth estate out of eighteen animals, six gave a positive reaction. Five of these reacting animals were raised on sterilized milk and had never come in contact with tuberculous animals. Besides, they had received their last protective vaccination only two months prior to the tuberculin test. On slaughter one of these five reacting animals was found to be free from tuberculosis. Roemer is therefore inclined to regard the positive outcome of the tuberculin test in these cases as due to over-sensitiveness. Concerning these favorable results obtained on the five estates above-mentioned, Hutyra points out that in addition to bovo-vaccination, hygienic prophylactic precautions were observed, and it is therefore hard to decide whether and to what degree the last mentioned measures contributed to the gratifying results.

Novak reports having immunized a number of cattle strictly in accordance with von Behring's directions. He injected from 1903 to May 1907, 332 calves in four different herds. In a fifth herd, which was entirely free from tuberculosis, he bovo-vaccinated twenty-seven calves with the object in this herd of determining whether the method of von Behring was really harmless, and whether it was not possible to cause through the vaccination tuberculosis. However, he found that this was not the case and that the vaccination itself was harmless. Of the 332 calves vaccinated in the four other herds a tuberculin test was made on 105 of these animals in 1905, and almost half of them, or 44%, gave a positive reaction. In order to verify the reliability of the tuberculin test, a number of the reacting animals were slaughtered, and it was found at the autopsy that the results were in complete conformity with the tuberculin tests. On account of these results Novak came to the conclusion that the method of bovo-vaccination is devoid of practical importance and that we are not able to protect through it cattle against natural infection with bovine tuberculosis.

Smith reports on experiments of thirty-five calves that were conducted under the auspices of the Massachusetts Society for promoting agriculture. He claims that it is possible for vaccination to produce resistance of longer and shorter duration, sometimes of a high degree depending somewhat on the animal to be vaccinated, as well as on the culture of tubercle bacilli used for the preparation of the Vaccine. He was able to obtain a better degree of immunity with fresh cultures of tubercle bacilli of the human type than with the method of bovo-vaccinating animals, as outlined by von Behring. He was never able to obtain a lasting immunity. Among his conclusions are the following:

(1) "Vaccination of calves with the human type of the tubercle bacillus is harmless. Cases in which injury are said to have resulted from it may have been due to other concomitant affections, among which pneumonia is probably the most common. Persons trying vaccination should first assure themselves that the culture they intend to use belongs to the human and not to the bovine type of the bacillus."

(2) "Vaccination with the human type of bacillus leads to a relatively high resistance to fatal doses of the bovine bacillus."

(3) "Vaccination with a carefully tested attenuated bovine bacillus may be as efficacious, even in a single injection, as the double vaccination with human bacilli."

(4) "The immunity conferred by vaccination as hitherto practiced does not appear to be satisfactory as regards degree or duration."

(5) "The immunity acquired by two vaccinations with human bacilli should be fortified by a subsequent injection of attenuated bovine bacilli."

(6) "The survival of human and bovine bacilli in the lungs and udders of calves vaccinated intravenously with them should be more definitely determined."

Haring reported before the American Veterinary Medical Association in 1910 some results in the testing of von Behring's Bovo-vaccine. Forty-five calves were used in the experiment. Twenty-two of these were treated with bovo-vaccine after the manner of von Behring, and twenty-three were kept as controls. Great care was taken to follow the method and technique prescribed by von Behring. Precautions were taken to prevent the error of vaccinating calves already infected, and during the first year the calves were reared with every possible precaution against tuberculous infection. At the end of eight months all the calves were tested with tuberculin and none reacted. The entire forty-five animals were subjected to infection by being placed in a pasture with five reacting cattle that were apparently in the advanced stage of tuberculosis. Within six months these five infected cattle had died of tuberculosis. During the following winter no tuberculous animals were associated with the young cattle. During the next Spring ten more reacting dairy cows were placed in the corral and pasture with the young cattle. Three of these cows were proved to have open tuberculosis by infecting guinea pigs from their feces and all were eventually found to be tuberculous by autopsy. All of the experimental animals were slaughtered and at autopsy, of the 22 vaccinated animals 10 were tuberculous, and of the 23 non-vaccinated cattle 14 were tuberculous. In a summary of the report they state, "Some immunity was produced by the use of von Behring's bovo-vaccine, two doses of 1 and 5 immunizing units respectively being used. This immunity did not remain to any appreciable extent after the third year."

EXPERIMENTS WITH FRESHLY PREPARED VACCINE OF LIVING HUMAN TUBERCLE BACILLI.

At the International Veterinary Congress in Budapest in 1905, Pearson presented a report of some experiments performed by him jointly with Gilliland. He claimed that it was possible to attain a higher resistance against tuberculosis in cattle by intravenous injections of living human tubercle bacilli suspended in normal salt solution. This resistance, he stated, depended in a degree on the virulence of the strain of tubercle bacilli used for the vaccinations as well as on the number of vaccinations given.

Thomassen further in his work recommended that vaccination should be performed three times at intervals of four weeks. The best method of vaccination was intravenous inoculations with slightly virulent freshly grown human tubercle bacilli. The dosage he recommended was 1 milligram for the first vaccination, 10 milligrams for the second vaccination and 20 milligrams for the third vaccination.

Hutyra reports upon the vaccination of three calves varying in age from three and a half to nine months, with Vaccine prepared from freshly grown cultures of the human type of tubercle bacilli. The method of vaccination was intravenous. The animals so treated he claimed showed a greater resistance to a test infection performed some time later than cattle bovo-vaccinated by the von Behring method.

Weber & Titze performed some experiments with seven cattle from eight to twelve months of age which were injected intravenously with 50 milligrams of human tubercle bacilli that had been grown on glycerin bouillon. In order to test the resistance of these animals some of them were inoculated intravenously and others subcutaneously with virulent bovine tubercle bacilli. The infection inoculations took place in some cases as early as five months and in others as late as two years after the vaccinations. It was found that the animals inoculated as late as a year after the vaccination showed more serious changes than ones which were infected only five to six months after immunization. One animal which had been inoculated intravenously two years after immunization died 21 days later, and behaved exactly like a non-immunized animal. They claim that it should also be mentioned that in course of other experiments that they conducted for the immunization against tuberculosis that some of the immunized animals when later tested with bovine tubercle bacilli, developed lesions of tuberculosis of the brain and its membranes, as well as of the eye and the sexual organs.

IMMUNIZATION WITH TAURUMAN.

Koch, Schuetz, Neufeld and Miessner tried to attain immunization of calves by a single injection of a large dose of tubercle bacilli of the human type. They believed that a single vaccination was more practical than a double vaccination. They conducted some experiments by which they claimed it to be possible to immunize cattle against highly virulent bovine tubercle bacilli by a single injection of 10 milligrams to 30 milligrams. The bacilli used for this purpose are to be grown on glycerin bouillon, and they recommend a culture of 30 to 40 days old be used. The bacilli are removed from the bouillon and dried between sheets of blotting paper. The mass of bacilli is removed from the blotting paper with the aid of a platinum spatula. From the dried mass of bacilli the exact quantity desired is weighed and then gradually ground in an agate mortar with 10 cc. of a physiologic salt solution. They recommend the injection of a Vaccine in the Dorsal Vein. The full immunity of the vaccinated calf is not established until three months after the vaccination.

This Vaccine was later prepared according to Koch & Schuetz's directions and was placed on the market in sealed glass tubes under the name of "Tauruman." It is in the form of an emulsion or suspension of live, human tubercle bacilli in salt solution. It was mentioned on the label that the vaccination is to be performed only on healthy animals and during the first few months of their life. Only a single intravenous injection of 10 cc. of Tauruman was recommended in each case. These same authors earlier in their work observe an immunizing action following two intravenous injections of an attenuated culture of bovine tubercle bacilli, the interval between the injections being eight weeks, the dose of Vaccine used each time being 10 milligrams. Later they used in addition to the attenuated bovine tubercle bacilli cultures of various strains of human tubercle bacilli in the preparation of the Vaccine. At the first injection some of the calves received intravenously 10 milligrams, others 20 milligrams of tubercle bacilli, suspended in either 5 cc. or 10 cc. of an .8% normal salt solution. At the second vaccination each calf received 50 milligrams of the same culture injected in the same manner. The interval between vaccinations was from one to two months. They note that following the first vaccination the temperature of the animal rose to 40 or 41° C. and remained at that point for several days, while following the second vaccination the temperature also rose but only for a few days and without disturbing the general condition of the animal. In order to test the immunity of these calves the authors injected intravenously into each animal 20 milligrams of a culture of bovine tubercle bacilli. It was found that one-fortieth of this dose would have been sufficient to produce a fatal miliary tuberculosis in a calf within 20 to 30 days. In 6 out of the 18 calves which had received protective vaccinations, the injections of the virulent bovine tubercle bacilli were made in about forty days following the second vaccination. Three of these calves withstood these infection injections, while the other three were found to be tuberculous at autopsy. The partial failure of these experiments was explained by the investigators as possibly due to too short a time between the second vaccination and the period of infection. Therefore, they did not make the control tests on the other calves until after twelve weeks had elapsed following the second protective vaccination, the results of which were that all the animals remained in perfectly healthy condition with the exception of two which showed comparatively minor healed lesions of old tuberculous processes.

Weber & Titze carried out experiments to test on herds Tauruman prepared according to the directions of Koch & Schuetz, in the same manner that they tested the bovo-vaccine of von Behring. The animals used in their tests were vaccinated with Tauruman at the end of three weeks. The calves came from stables which were under veterinary supervision. Calves which showed following the intravenous injection of Tauruman a temperature reaction were discarded from the experiment. The immunity conferred upon the animals by the injection with Tauruman was tested by intravenous and subcutaneous injections of bovine tubercle bacilli. Also, some of the animals were exposed to natural infection to tuberculosis and some were exposed to inhallation and feeding of bovine tubercle bacilli. For these injections, inhallations and feedings the same culture of bovine tubercle bacilli was used as was employed in the tests of cattle treated with bovo-vaccine. The tests of the immunity of the animals were made from four to nine months following the vaccination with Tauruman. These authors report that all the animals vaccinated with Tauruman and which were subsequently tested in different ways, not one was found to be free from tuberculosis. They state, however, that a transitory increased resistance was distinctly apparent, especially when the method of infection was by inhallation or feeding.

Weber & Titze also made further experiments with Tauruman in their practice. They vaccinated during the years 1906 and 1908, 41 animals from two to seven days old on a farm on which tuberculosis was very prevalent. In January, 1908, twenty of these animals which had been vaccinated with Tauruman were tuberculin-tested with the result that 19 or 95% gave a positive reaction. In March, 1909, 23 of the animals which had been vaccinated with Tauruman a year or more before, were tuberculin tested with the result that 20 or 86.95% gave a positive reaction. Among the animals slaughtered from 1 year and 2 months to 3 years and 1 month after the vaccination with Tauruman, 6 or 50% were found free from tuberculosis. However, the authors point out that the apparently satisfactory result is due not so much to Tauruman vaccinations in this instance as it was to the elimination of the chances for infection. They state that a cow afflicted with tuberculosis of the udder was removed from the herd and the calves were fed on boiled milk. Zwick & Titze state that the final results of the experiments of the above men with Tauruman vaccinations can be summed up in the sentence "That an appreciable difference between the bovo-vaccine and the Tauruman Vaccine as regards their immunizing virtue does not exist." Meissner has done considerable work with Tauruman Vaccine and his examination has been that one is able to produce absolute immunity in laboratory experiments, but is unable to obtain immunity in cattle when they are exposed to natural infection.

HEYMANS METHOD OF TUBERCULOSIS IMMUNIZATION.

Heymans announced a new method of producing immunity in cattle which differs somewhat from any of the methods previously described. It consists in the introduction underneath the skin of reed bag containers filled with live virulent human or bovine tubercle bacilli in the dry form. The method of introducing these reed bag containers under the skin is with a special trocar devised for this purpose. An incision from 2 to 3 cmm. long is made in the skin behind the shoulder and the trocar containing the reed bag is introduced through this incision and the wound is closed by a special metal clamp. In order to protect the reed bag against crushing, it is further enclosed before introduction in a gelatine capsule. The advantages of this method according to Heymans is that cattle of any age can be vaccinated and the vaccinations can be repeated as often as desired. He further claims that cattle already afflicted with tuberculosis may be vaccinated in this manner. The object of the reed bag is to permit the exosmosis of the products of the tubercle bacillus as well as endosmosis of the tissue fluids of the body. It is assumed that from the reed bags there takes place an impregnation of the whole body of the animal with the specific soluble products of metabolism of the tubercle bacilli and that thereby immunity results. He claims as a rule no reaction takes place at the point of vaccination. Occasionally there may be a slight suppuration, which, however, does not impair the general condition of the animals vaccinated. It has been found that animals vaccinated in this manner will react to tuberculin in from 10 to 14 days following vaccination.

According to Eber he found a few weeks after the introduction of the reed bag that they became surrounded with a thick, grayish yellow mass in which a large number of tubercle bacilli were present.

Heymans is of the belief that the immunity is of about a year's duration, and states that he believes that it is possible to eliminate tuberculosis in three to four years in 80% of infected stables if the whole herd is re-vaccinated every year. In such stables, however, he recommends besides vaccination the use of prophylactic measures. At the International Veterinary Congress at The Hague, Heymans stated that in more than a thousand herds a total of 12,000 animals had been vaccinated. At the autopsy of 1,000 animals that were in a healthy condition at the time of vaccination and had later not given a positive reaction to tuberculin, they were found to be free from tuberculosis. Further, that vaccinated tuberculous animals which after vaccination did not continue to react to tuberculin, showed old tuberculous lesions in a state of retrogression.

The commission appointed by the Belgian Government to test the value of Heymans method, reports in 1912 in the Annal. de med. vet.

that it is their opinion that the vaccination according to this method is not able to protect cattle, either against natural or artificial infection, and is therefore of doubtful practical value.

Mohler and Schroeder of the U. S. Bureau of Animal Industry in 1910 reported upon the immunization of cattle against tuberculosis, giving the results obtained by them with the von Behring method, the Heymans method and the Pearson method. The latter method is the one suggested by the late Dr. Leonard Pearson and was used largely in connection with the experiments heretofore reported in Part I of this Bulletin. They found in a comparison of the methods that 88.8% of the animals immunized by the Pearson method were successfully protected for a period of approximately two years, while only 66 2-3% were successfully protected by the von Behring method. It was found that the control animals used in conjunction with the vaccinated animals in testing the value of these two methods of immunization contracted tuberculosis to the extent of 85 5-7% or in other words only 14 2-7% escaped infection as compared with the above percentages of immunized animals. They inoculated 12 cattle and 10 hogs with capsules prepared after Heymans' method. The method of testing immunity conferred by these methods was by associating the vaccinated animals with tuberculous cattle. Among the hogs vaccinated according to Heymans' method, one contracted generalized tuberculosis from the vaccinations and one died prematurely as the result of an injury. When the remaining eight hogs with their ten controls were killed after an exposure of natural infection of eight months to a year, all of them were found to be affected with tuberculosis, and the lesions in the vaccinated animals were in no respect different from those found in the checks. Hence the authors conclude that it is very clear that Heymans' method is absolutely worthless for hogs. Among the twelve cattle vaccinated, according to Heymans' method, one died prematurely and the remaining eleven when killed showed lesions of tuberculosis. One of the vaccinated animals showed tuberculosis directly traceable to the capsule with which it was inoculated for protection. They, therefore, draw the conclusion that Heymans' capsule method of bovo-vaccination is inefficient. These authors also point out the fact that the vegetable sac in which the tubercle bacilli are enclosed, is capable of permitting bacteria to pass through. This they proved by introducing under the skin of sheep capsules in which anthrax bacilli were contained. These authors conclude in summing up the three methods that "No system of bovo-vaccination has reached the stage at the present time that justifies its use in common practice."

THE ADMINISTRATION OF TUBERCULOSIS VACCINE SUBCUTANEOUSLY AND THROUGH THE INTESTINAL TRACT.

von Baumgarten reports upon a method of immunizing cattle against tuberculosis by the subcutaneous injection of the animals with a single dose of living human tubercle bacilli. In his experiments he used almost exclusively tubercle bacilli having the cultural and morphological characteristics of the human type, which he stated were virulent for rabbits. In the course of his experiments he cites that cattle immunized for 4 to 4½ years according to this method, have not shown any local or general reaction worth mentioning after having received seven test inoculations with doses of bovine tubercle bacilli which were fatal for control animals. The vaccinated animals, he reports, never reacted to tuberculin. At the time of slaughter some of them were found to be completely free from tuberculosis, while others were comparatively free from lesions of the disease. The advantage of this method according to the author is that the tubercle bacilli used in the Vaccine became localized at the point of injection or at the nearest lymph glands, and do not pass into the blood stream or internal organs. The objection to the method which has been raised by some investigators is the fact that the tuberculous node arising at the point of vaccination might soften and break down and the tubercle bacilli thereby become disseminated, and be a source of danger for man in the consumption of the milk or meat of such an animal. In von Baumgarten's opinion, this danger can be avoided by using a smaller dose of the dry culture of tubercle bacilli. He even suggests that a dose of 10 milligrams would be sufficient for very young calves to make them resistant against subcutaneous infection with virulent bovine tubercle bacilli.

Lignieres reports having used the subcutaneous method of vaccination in the fight against tuberculosis of cattle in Argentine. He used an emulsion of live human tubercle bacilli of the Arloing-Courmont homogeneous culture in doses of 50 to 100 milligrams. According to his results these cattle were found to be just as immune against test injections as cattle bovo-vaccinated according to the von Behring method. Hutyra tried some experiments with the subcutaneous method of vaccination, believing that with such a method the tubercle bacilli of the Vaccine would remain viable for a long time at the point of vaccination, and thereby bring about a more lasting and active immunity. From his experiments it appears that the resistance of cattle which were given a single subcutaneous injection of vaccine prepared from human tubercle bacilli, was considerably increased against natural infection. However, this high degree of resistance obtained immediately after the vaccination, decreased after six months. Hutyra suggests that the intravenous protective vaccination which has to be performed twice, could very well be replaced by a single subcutaneous vaccination of 50 to 100 milligrams of fresh cultures of human tubercle bacilli.

Weber & Titze obtained in two experiments with the subcutaneous method of vaccination entirely negative results. Both animals that had been vaccinated died in 14 and 28 days respectively after they had been inoculated with 10 milligrams of tubercle bacilli of the bovine type. This infection took place eight months after the vaccination. These authors also set forth objections to the subcutaneous method in practice on the ground that freshly grown human tubercle bacilli are used and an abscess at the point of vaccination occurs, which very often opens and discharges pus containing tubercle bacilli, thereby being a source of infection.

Calmette and Guerin made attempts to obtain immunity against tuberculosis by introducing attenuated or avirulent bovine tubercle bacilli into the stomachs of calves and goats by the means of a stomach tube. Their method of attenuating the bovine tubercle bacilli was by boiling for five minutes or by heating for the same length of time at 70° C. The results of their experiments in brief were that when tubercle bacilli of the bovine type that had been killed or whose virulence was decreased, were introduced into the stomach of calves and goats in doses of 50 milligrams and 45 days later 25 milligrams, that these animals were afterwards capable of withstanding an infection with 50 milligrams of fresh tubercle bacilli by ingestion. This doses of fresh tubercle bacilli was invariably fatal for control animals.

Other attempts have been made to immunize cattle against tuberculosis by the injection of killed tubercle bacilli. These results have not been satisfactory and no hope can be entertained of obtaining a serviceable degree of immunity by the use of dead tubercle bacilli in the preparation of the Vaccine.

THE VALUE OF TUBERCULIN IN THE PRODUCTION OF IMMUNITY.

McFadyean has reported upon four cattle in which he gave large and repeated doses of tuberculin as well as emulsions of tuberculous material and cultures from various sources. Two of the four animals had given a positive tuberculin reaction prior to the experiment and were no doubt tuberculous when the experiment began. The other two animals gave no reaction to the tuberculin test and were evidently free from the disease. One of the animals which was tuberculous at the beginning of the experiment in 1899, received nine injections of various amounts of tuberculin, and was then given four injections of 10 cc. each, followed by five injections of 20 cc. each or a total of 150 cc. of tuberculin. Approximately two weeks following the last injection of tuberculin the animal was inoculated intravenously with 2 cc. of a heavy suspension of tuberculous tissue from the mesenteric gland of a horse. Fifteen weeks after the inoculation with the virulent tuberculous material, this animal was killed and was found to contain but one tubercle the size of a pea and completely calcified in a mesenteric gland. Two control cattle inoculated with an equal dose of the same tuberculous material were killed approximately eight weeks after the injection and both were found to be extensively tuberculous. The second animal, which was also tuberculous at the time the experiment began, received four injections of 1 cc. each of tuberculin then 1.5 cc. intravenously of an emulsion of the tissues of a rabbit infected from an ox. The animal was then given further tuberculin injections amounting to 20 cc. at each injection for six separate injections and then 10 cc. for two different times. The animal was then inoculated intravenously with 2 cc. of a heavy emulsion of tuberculous tissue from a rabbit inoculated from an ox. This was followed by four injections of 1 cc. each of tuberculin, then 2 cc. intravenously with a heavy emulsion of tuberculous tissue from a horse. Then two injections of tuberculin followed by 5 cc. intravenously of a heavy emulsion of tubercle bacilli from a culture that was virulent for a rabbit, followed by 1 cc. of tuberculin. Then 5 cc. intravenously of a heavy emulsion of tubercle bacilli from a culture virulent for guinea pigs. Then 1 cc. of tuberculin followed by 5 cc. intravenously of an emulsion of tubercle bacilli the same as the previous injection. Then two injections of tuberculin followed by 10 cc. intravenously of an emulsion of tubercle bacilli of a culture virulent for a pony and moderately virulent for a calf, in doses of 2 cc. followed by two injections of tuberculin. The animal died suddenly and on autopsy the kidneys were found to be extensively tuberculous, and the piamater at the base of the brain showed many tubercles. Slight lesions were also found in several lymphatic glands. The remaining two animals which were free from tuberculosis at the beginning of the experiment, were inoculated intravenously from 7 to 11 times during a period of from two to three years with emulsions of tuberculous material and with cultures from various sources. One of these animals received between the first and second injection of emulsions of tuberculous tissue, 19 injections of tuberculin, and both animals received between each injection of tuberculous material injections of tuberculin from one to four times. The first injection of tuberculous tissue for these two animals was from a fowl. These two cattle died of tuberculosis after from two to more than three years from the beginning of the experiment and the most interesting part is to note that the chief lesions were in the kidneys or the brain or its covering membranes. The cerebral lesion appears to have been the immediate cause of death in each instance. There can be no doubt that these animals were remarkably resistant to tuberculosis, because they lived for some months and years after repeated inoculation with large quantities of material proved virulent for cattle. At the time of the publication of these experiments McFadyean concludes "It therefore appears to be justifiable to conclude that whatever may have been the degree of natural immunity possessed by these cattle, it was much increased by the successive inoculations to which they were subjected. The immunity was not absolute, but it may be doubted whether a degree of resistance that will merit this term is obtainable by any method in cattle."

In 1901 Pearson & Gilliland published the results of an experiment for the purpose of determining the influence of Koch's Original Tuberculin upon the resistance of cattle to tuberculosis. In this experiment they used four calves. Each animal was carefully tested with tuberculin before it was admitted into the experiment. Two of the cows were given daily subcutaneous injections of 5 cc. of Concentrated Tuberculin for ten days. Sixteen days following the last injection of tuberculin, each of the four cows in the experiment was fed daily 100 grams of tuberculous lung tissue from a cow for ten days. The two animals that had received preliminary injections of tuberculin were given subcutaneously 15 cc. of Concentrated Tuberculin each day during the progress of the feeding of the tuberculous material. The two cows which had not received daily preliminary injections of tuberculin, received no tuberculin whatever. The animals were killed approximately three months following the feeding of the tuberculous material. The two animals that had received treatment with tuberculin, one showed upon post-mortem examination lesions of tuberculosis in the post-pharyngeal and mesenteric lymphatic glands, while the other animal showed no lesions of tuberculosis excepting in the mesenteric lymphatic glands. A few of the glands of both the small and large intestine showed small areas of caseation. The two control animals which had received no tuberculin whatever, were killed at approximately the same time. One animal exhibited lesions of tuberculosis in the right lung, in the bronchial and mediastinal lymphatic glands, the post-pharyngeal and inter-maxillary lymphatic glands and in the mesenteric lymphatic glands, while the other animal showed lesions of tuberculosis in both lungs, bronchial, mediastinal and post-pharyngeal lymphatic glands, as well as the lymphatic glands of the mesentery. The lesions in the control animals were more widely distributed and more advanced than in the animals that had received large quantities of tuberculin. The authors conclude that "From this it would appear that subcutaneous injections of the toxin of the tubercle bacillus had had some influence in increasing the resistance of these two animals to feeding tuberculosis."

EXPERIMENTS FOR THE PRODUCTION OF IMMUNITY WITH AVIAN TUBERCLE BACILLI.

Grancher and Ledoux-Lebard announced in 1891 that they had made some tests with the object in view of the production of immunity against tuberculosis by the use of avian tubercle bacilli. They injected rabbits intravenously with tubercle bacilli from poultry, but did not find that the animals developed any marked degree of immunity.

This subject has also been studied by Hericourt & Richards, Trudeau, Bades, Courmont & Dor, Paterson and others, but the results of their experiments are in no way encouraging and it would appear that they did not obtain sufficient immunity to be of any definite value. Roemer conducted some experiments upon cattle with tubercle bacilli obtained from chickens, but instead of obtaining a marked degree of immunity the animals showed an over-sensitiveness. The method was abandoned by him as being too dangerous.

EXPERIMENTS FOR THE PRODUCTION OF IMMUNITY WITH TUBERCLE BACILLI OF COLD-BLOODED ANIMALS AND OTHER ACID-FAST BACILLI.

Friedman has reported upon some experiments on cattle with tubercle bacilli which are supposed to be harmless for warm-blooded animals. The experiment consisted of two cattle, one of which was vaccinated once intravenously with tubercle bacilli from a turtle. After two and a half months, in order to test its immunity, this animal was inoculated intravenously with a highly virulent culture of bovine tubercle bacilli. At the autopsy performed approximately three months later there were found in the lungs of this animal hard nodules which did not contain any tubercle bacilli that were virulent for guinea pigs. According to the author these nodules were caused by tubercle bacilli from the turtle and that the condition should be considered as a local one in the process of healing. The control animal, which had received no injections of tubercle bacilli from the turtle, was found to be afflicted with an extensive acute tuberculosis. The other animal in the experiment received repeated injections of Vaccine prepared from the same culture. This animal the author states did not react to repeated injections of large amounts of tubercle bacilli of bovine type which were administered following the vaccinations. An autopsy was not performed on this animal because it was used for other purposes.

Libbertz & Ruppel in contrast to Friedman's work have proved by a number of experiments that intravenous injections of turtle tubercle bacilli are by no means harmless for warm-blooded animals, but on the contrary are capable of causing fatal intoxications and also producing organic changes. Animals which they had vaccinated with turtle tubercle bacilli and died following the vaccinations, showed acute edema of the lungs and hemorrhages of the serous membranes.

Orth reports upon some experiments conducted by him in which he was able to show that guinea pigs that had been vaccinated with turtle tubercle bacilli would live longer when later infected with virulent tubercle bacilli than non-vaccinated control animals. Weber & Titze report upon the inoculation of seven cattle approximately seven months old with cultures of tubercle bacilli from cold-blooded animals. The animals were given intravenously 50 milligrams as the initial dose, two months later 100 milligrams and five months later 100 milligrams of the same culture. When the animals were subjected, four months after the last injection, to a test infection with bovine tubercle bacilli, no essential difference was noted as compared with the non-vaccinated control animals. All of them without distinction became tuberculous.

It is hardly possible from these experiments to draw any definite conclusions as to the value of tubercle bacilli from cold-blooded animals in the production of immunity against tuberculosis in cattle Koch, Schuetz, Neufeld and Miessner found that serum of goats which had been immunized against tuberculosis, was capable of agglutinating not only tubercle bacilli, but the other acid-fast bacilli. They therefore decided to start experiments to prove whether acid-fast bacilli were capable of producing immunity against tuberculosis. They made use of goats to which they administered timothy bacilli, pseudo bovine bacilli, and cultures of material of the tubercle bacillus of the blind worm. It appears that the vaccines produced injurious after-effects and the results were not satisfactory in any way.

Moeller has also reported upon some experiments on guinea pigs vaccinated with the timothy bacillus, the grass bacillus and the pseudo bovine bacillus, but even by repeated vaccinations he was not able to bring about any appreciable degree of immunity.

Weber & Titze report upon an animal that had received three vaccinations with a culture of timothy bacilli and was tested two months after the last injection by an intravenous inoculation of five milligrams of bovine tubercle bacilli. This animal showed only a slight resistance. They report further that animals which have been vaccinated by single injections with 50 milligrams of fish tubercle bacilli, of blind worm tubercle bacilli as well as timothy bacilli, showed no increase whatever in their resistance against tuberculosis when tested five months later.

KLIMMER'S METHOD OF PROTECTIVE VACCINATION.

Klimmer's method consists in the using of tubercle bacilli in the preparation of the Vaccine which are non-virulent for man as well as animals, and according to his statement has lost all virulence for guinea pigs. He has prepared two Vaccines. The first he designates as TH and the other AV. The first Vaccine is prepared by attenuating the human tubercle bacillus by heating the same to 52 or 53° C. or, as he states, to a point just below the virulence for guinea pigs. This Vaccine is manufactured by a firm in Dohna and is sold under the name of "Antiphymatol." The second Vaccine designated AV is prepared from tubercle bacilli which are isolated from the organs of cold-blooded animals (Salamanders). These animals had been repeatedly treated with human tubercle bacilli that had passed through several Salamanders before they were used for the preparation of the Vaccine. It is claimed by Klimmer that these bacilli are avirulent for man, mammals and birds. He recommends that all milch cows should be vaccinated exclusively with this second Vaccine known as AV. The dose recommended is 5 cc. of a Watery Suspension and the vaccination is performed subcutaneously. Cattle of any age may be vaccinated. He claims protective as well as curative value for these Vaccines. Animals that are free from tuberculosis are to be vaccinated twice in the first year and those that are suffering from tuberculosis are to be vaccinated four times with an interval of three months. The vaccinations should be repeated on all animals every year. The author states that the immunity is well developed after two months and remains at the same height for almost nine months. He further recommends in the application of the Vaccine to an infected herd, that the best of hygienic measures be observed, that all calves be fed on boiled or pasteurized milk, and that when the same is not possible that they should receive milk from cows known to be free from tuberculosis. Klimmer later withdrew the first Vaccine known as TH and recommended for protective vaccination in practice only the second Vaccine AV. It is stated that 43 cattle vaccinated after Klimmer's method when killed later were all found free from tuberculosis. The slaughtering took place in the case of 16 cattle during the first five months following the first vaccination, in 8 cattle one to two years, in 7 cattle two to three years, and in the remaining animals after a longer period. Klimmer stated at the international Veterinary Congress at The Hague in 1909 that 20,000 cattle had been vaccinated according to his method up to that time. Against the method as advocated by Klimmer, several investigators have brought forth objections. Eber claims the Vaccine prepared by attenuating human tubercle bacilli by heat, known as the 'Thermic Method," TH, does not lose its virulence for guinea pigs in all cases. Weber & Titze state that the Vaccine, TH., is not always as harmless as claimed by the originator. Weber & Titze surmise that the Vaccine prepared from the virulent tubercle bacilli, AV, are not modified human tubercle bacilli, as their investigations appear to show that the bacilli are acid-fast saprophytes, as it appears that these bacilli don't grow well at incubator temperature, but grow best at room temperature and on ordinary culture media. Their cultures presented appearance different from that of tubercle bacilli and were completely avirulent for small laboratory animals as well as for frogs and salamanders. They obtained acid-fast bacilli from the intestinal contents of salamanders and compared them with these avirulent tubercle bacilli, and they were very similar. Weber & Titze made further experiments on Klimmer's method on four calves approximately three to four months of age that had been found to be free from tuberculosis by the tuberculin test. The first two animals received a dose of avirulent tubercle bacilli subcutaneously and were vaccinated about three months later in the same manner. Five months after the second vaccination these animals were exposed to natural infection by association with a cow suffering from pulmonary tuberculosis. The two control animals were exposed in the same way at the same time. They state that at the autopsy no marked difference could be noticed in the degree or the extent of the disease between the vaccinated and control animals. Two animals were also vaccinated after Klimmer's method with attenuated human tubercle bacilli and the result was the same.

Edelmann has made numerous tests according to the Klimmer procedure. A total of about one thousand animals were vaccinated. The hygienic measures recommended by Klimmer were not followed in these tests. About three hundred of these animals were vaccinated by the intravenous method first recommended by Klimmer before changing to the subcutaneous method. Of forty animals that were killed after having been vaccinated by the intravenous method, twenty-four animals were found tuberculous. The interval from the time of the last vaccination until the slaughter of the animal was from 14 days to 3¼ years. Of five cattle vaccinated the first time intravenously and then with two or more subcutaneous vaccinations, four were found afflicted with tuberculosis. He found that of twenty animals vaccinated entirely by the subcutaneous method, that three animals which had been vaccinated three times, one was found infected; fourteen animals which had been vaccinated twice, ten were found infected, and three animals which had been vaccinated once, none of them contained lesions of tuberculosis. He further found that when the tuberculin test applied to vaccinated animals, positive reactions were obtained in from 45 to 51% of cases. According to the author, he does not feel that it is possible to protect cattle against natural infection with tuberculosis by this method without the simultaneous use of hygienic measures. Klimmer raises the objections to these tests of Edelmann that the necessary hygienic precautions which he recommends with the use of his Vaccine, were not sufficiently observed, and no special attention was paid to the protection of calves against milk infection. He further claims that in Edelmann's experiments the tuberculin test was not applied prior to the vaccinations and that in cases of several animals the prescribed yearly re-vaccinations were not carried out or else were done at a later date than recommended by him.

THE PRODUCTION OF IMMUNITY WITH TUBERCLE BACILLI ATTENUATED BY CHEMICALS.

Levy as well as Blumenthal and Marxer have made attempts to kill tubercle bacilli with chemicals and at the same time preserve their antigen quality as far as possible. They afterwards employed tubercle bacilli treated in this manner for immunizing purposes. For attenuation they used 25% solutions of galactose, 10 and 25% solutions of urea and 80% solution of glycerin. These authors claim to have been able to show that tubercle bacilli treated in this manner are capable of immunizing such animals as guinea pigs and rabbits against human and bovine tubercle bacilli.

Noguchi made some attempts to produce immunity against tuberculosis with the use of tubercle bacilli which had been subjected to Oleic Acid bases like Natrium Oleinicum, Neurin and Ammonium Oleate. Guinea pigs which had been injected with bacilli so treated, showed either a complete or partial resistance against a subsequent injection with a virulent culture of the same strain of tubercle bacilli.

Zenner treated some tubercle bacilli with a solution of Oleate of Sodium 1 to 60. The bacilli were thoroughly shaken in this solution and then heated for a long period of time, and afterwards the solution was filtered. The filtrate was used for the inoculation of guinea pigs. His results showed that the use of this filtrate appeared to prolong the life of animals suffering from tuberculosis.

Broll carried on some experiments in which he inoculated guinea pigs with an emulsion of tubercle bacilli prepared as follows: Two grams of tubercle bacilli were supended in 100 cc. of a solution of Natrium Oleinicum. These were shaken up in an apparatus for six days at a temperature of 37° C. and then heated on a water bath at 70° C. and again placed for three days in a shaking apparatus. Tests of this product showed that guinea pigs inoculated with the same failed to develop tuberculosis. With the Vaccine prepared in this manner Broll made immunization tests on two calves. One calf received an injection of 10 cc. and after six weeks another injection of 20 cc. At the site of injection a swelling developed which disappeared in a few days. Four weeks after the last injection the animal was given intravenously two and one-half milligrams of virulent bovine tubercle bacilli. At the same time a control calf was inoculated with the same dose and in the same manner. When the immunized calf was killed ten weeks later, only a slight degree of tuberculosis of the bronchial and mediastinal lymphatic glands as well as of the lungs and of the kidneys was found, while the control calf was afflicted with generalized tuberculosis fifty days after the infection. The second test calf received at intervals of four weeks three subcutaneous injections of this bacterial preparation, the dose being 10 cc. for the first two injections and 20 cc. for the third injection. The test infection of this animal was performed in the same manner as with the first calf. The result was still more favorable. Besides a sterile abscess at the site of injection there was found only a tuberculous nodule of the size of a pinhead on the pleura and a small caseous focus in one of the bronchial lymphatic glands.

B. The Injection of Domestic Animals with Tuberculin.

In human medicine a very marked reaction took place against tuberculin after a short period of tremendous enthusiasm following its announcement to the profession in 1890 by R. Koch as a remedy against tuberculosis and at the same time as a means of diagnosing the disease. This product has established for itself a recognized and gradually more and more important place in Veterinary Medicine, for the reason that Veterinarians early recognized its value for diagnostic purposes and paid little or no attention to the alleged therapeutic value of tuberculin.

The first experiments to establish the value of Old Tuberculin in the diagnosis of tuberculosis in cattle were made in 1890 and 1891. It is reported that Gutmann successfully used some of Koch's Old Tuberculin in doses of 0.2 cc. and 0.3 cc. in the Veterinary Institute of Dorpat on three tuberculous cows and two healthy bulls. About the same time A. Stricker injected 1 cc. of tuberculin in four cows which were suspected of tuberculosis. In all these cases the experiments were successful.

Following these brief experiments a systematic investigation of the value of tuberculin was made by Roeckl, Schuetz and Lydtin at the request of the Imperial Health Department of Germany. There were 133 cattle injected with tuberculin of which 80 reacted. At time of autopsy 67 of the 80 reacting animals were found to be tuberculous. Of the 53 animals which did not react, 5 were found to be tuberculous at time of autopsy. It is further stated that four of the five animals which did not show a reaction and were found tuberculous had a high temperature before the injection. The authors draw the conclusion that in cases under investigation tuberculin was found useful for diagnostic purposes not only in animals which were more or less suspected of tuberculosis, but also in animals which to all outward appearances were in a perfectly healthy condition.

Furthermore, tuberculin was found to be a particularly delicate re-agent in animals which were afflicted with only small latent calcified tubercles, these lesions often being overlooked in the ordinary examination of cattle at the abattoirs.

The experimenters afore-referred to found that doses of tuberculin of 0.5 cc. were sufficient for all initial tests. With smaller doses the reaction was in general less marked as was also the case when a second injection was given following the initial injection within a short time. The influence of age, sex or weight on the intensity of the reaction was not apparent during the experiments. No ill effects were noted with doses of 0.5 cc. of tuberculin injected several times. A reduction in the secretion of milk was noticed which was attributed to the fever and lessened appetite of the animal during the period of reaction, as well as to the frequent disturbance of the animal from taking the temperature measurements. They found that the increase of temperature reached its maximum mostly in 15 hours when the injections were made in the evening. They note that suspicious or positive reactions may be obtained in some other conditions besides tuberculosis and such conditions as pulmonary gangrene, liver abscess, actinomycosis, inflammation of the mammary gland as well as the glands of the intestines. They further state that the action is to be considered as positive beyond a doubt when the animal shows an increase of temperature of at least 2.2 F. and the maximum temperature of the animal reaches 104° F. In animals which show an elevated temperature at the time of injection, the use of tuberculin is doubtful.

In 1891 Prof. Bang of Denmark published a very extensive work on the importance of tuberculin for the diagnosis of tuberculosis in cattle and pigs. He came to the conclusion that this product is a very delicate re-agent for the detection of tuberculosis of cattle, as it produces a typical reaction even in cases in which the disease is insignificant and in which it is impossible to demonstrate it by any other means. He believes further that it has great value in the diagnosis of tuberculosis in pigs and does not believe that it is capable of aggravating an existing tuberculous condition except in cases of animals suffering with advanced or generalized tuberculosis.

The experiments with tuberculin in the years 1890 and 1891 and up to February 1892 were collected by A. Eber. In his report twenty-five veterinarians had tested 247 cattle. In 134 of the animals a distinct reaction was obtained, while in the remaining 113 animals no reaction was noted. At time of autopsy 115, or 85.82%, of the 134 reacting animals showed distinct lesions of tuberculosis.

Of the 113 animals which did not react, 101, or 89.38%, were found to be free from tuberculosis at autopsy. The 12 remaining animals which did not react, representing 10.62%, were found to contain lesions of the disease. The report does not state whether any of the animals in this experiment were afflicted with the disease to such an extent that it could be clinically demonstrated.

In a collection of all the cases reported up to 1895 which were tested with tuberculin and in which the experiment was controlled by autopsy, A. Eber found that out of 563 cases in which a positive reaction had been obtained from the injection of tuberculin that 489 animals or 86.86% were tuberculous, while in 74 animals or 13.14% no lesions of tuberculosis could be found at time of slaughter. It was recommended by Eber at that time that the best dose for animals of average weight is 0.4 cc. to 0.5 cc. of Old Tuberculin diluted with nine to ten times the amount with a ½ of 1% solution of carbolic acid. The injection is to be made preferably on the lateral surface of the neck and either in the morning or late in the evening. The characteristic reaction appears in from 6 to 18 hours after the injection and lasts from 3 to 12 hours. Temperature measurements should be taken every two hours for the first six hours following the injection and then every hour from the sixth to the thirteenth hour.

Early in the use of tuberculin A. Eber conducted an experiment on a farm in which he divided the animals according to the reaction to tuberculin. The animals were divided into four groups, as follows:

Group A comprised such animals that gave no reaction.

Group B comprised all cases which, although they gave a typical reaction, did not present any other clinical manifestations of tuberculosis, and particularly had no cough and no enlargement of the supra-mammary glands.

Group C comprised all cases which showed a typical reaction and had no other manifestations of the disease except a slight cough.

Group D comprised all animals which gave a distinct tuberculin reaction and exhibited other marked clinical signs of tuberculosis.

He further recommends that a herd divided in this manner that the four groups should be kept separate from each other as far as possible, and that they should especially be kept in separate stables, and that for breeding purposes only cattle in Group A and only in exceptional cases certain animals from Group B should be used. The animals in Group D should be destroyed as soon as conditions permit.

In 1896 Prof. Bang of Denmark published a treatise upon the results of his method of elimination of tuberculosis in cattle which is based largely upon the use of the tuberculin test. It consists mainly in the isolation of the cattle showing a positive reaction from the non-reactors and in slaughtering of all the animals that are manifestly diseased. To further prevent the infection of the calves the milk should be boiled and the stables of the healthy animals thoroughly disinfected. The healthy animals should be tuberculin-tested twice a year to ascertain if any new cases have developed.

It was observed by Hess that animals which clinically showed the least manifestation of the disease gave the strongest reaction, while animals which were in poor condition, under weight or were in an advanced stage of tuberculosis, the reaction was often questionable and sometimes missing. He further observed that on those animals which he tested with tuberculin, all of them died of acute Miliary Tuberculosis after the injection, and therefore believes that latent tuberculosis may become active after tuberculin injections.

It may be stated that tuberculin found general recognition as a diagnostic agent through a resolution offered and passed at the Sixth International Veterinary Congress at Berne, Switzerland, in 1895. It was proposed by Bang and Nocard and was as follows: "Tuberculin is a very valuable diagnostic means and can render the greatest service in the fight against tuberculosis. There are no grounds to warn against its general use for fear of an aggravation of the pre-existing disease."

In judging by the cases reported up to that time, it seemed that in using the tuberculin test, even with the technique that was recommended, there was a relatively high percentage of mistakes in diagnosis. This percentage varied from 11 to 16. However, this percentage was considerably more favorable in 515 tuberculin tests which were verified by autopsy and which Bang reported in 1896. Of the 515 animals tested the diagnosis was found to be correct in all except 50 animals which represented a percentage of 9.7. In 1897 it was reported by Voges that he had collected all of the cases in which tuberculin tests were made up to that time and which were verified by autopsy. In 7,327 cases the diagnosis was found to be wrong in only 204 animals of 2.78%. Mohler of the U. S. Bureau of Animal Industry reports upon 24,784 animals tuberculin-tested and reacted in the United States between 1893 and 1908. The diagnosis was confirmed by autopsy in 24,387 cases or 98.13%. Therefore, he states that the subcutaneous tuberculin test enables us to make a definite diagnosis in at least 97% of cases and that tuberculin is a very important factor in the elimination of tuberculosis of cattle.

INTERPRETATION OF TUBERCULIN TESTS.

There is considerable difference among those who have had experience with the tuberculin test relative to the increase in temperature following the injection of tuberculin that would be considered a conclusive positive tuberculin reaction. This is evidenced by the abstract of works as follows:

Nocard considers a rise of temperature of 1.5° C. a clear distinct reaction, while a rise in temperature from 0.9° C. to 1.4° C. is considered doubtful and any rise below 0.9° C. is of no consequence. In Belgium, according to the Government decree of October 31, 1895, animals showing an increase of temperature of 1.4° C. above normal are barred from importation as tuberculous animals, and all animals which show a rise of temperature from 0.8° C. to 1.4° C. are rejected as suspicious.

Ostertag believes that not all cases of Tuberculosis are detected if an increase of temperature of 1.5° C. is insisted on as evidence of the animal being afflicted with Tuberculosis. He further states that the highest normal temperature of a calf during the first six months of life does not exceed 40° C., while that of an older animal does not go above 39.5° C. In his opinion all animals should be considered as suspicious of being afflicted with tuberculosis which show after the tuberculin injection a temperature in excess of 39.5° C. in grown cattle and in calves an excess of 40° C. He would also include in this class all animals whose highest temperature after the tuberculin injection was at least 0.5° C. higher than the highest temperature before the tuberculin was injected.

Hutyra considers as evidence of a positive reaction for animals older than six months: (1) A rise in temperature of 1.5° C. or more and over 40° C. when the difference amounts to at least 0.5° C. (2) A rise in temperature of from 1° C. to 1.4° C. with organic reaction. On the other hand, he claims we have no reason to make a diagnosis of Tuberculosis when the rise in temperature amounts to 1.4° C. at the utmost and has not exceeded 39.5° C., and at the same time no organic reaction was noticed provided a careful clinical examination of the animal shows no evidence which would justify a suspicion of the presence of Tuberculosis. He further believes that in the case of calves younger than six months only a rise of temperature above 40.5° C. is to be considered as evidence of a positive tuberculin reaction.

Johne points out repeatedly that we must not consider a positive reaction incorrect when we are unable to demonstrate a focal lesion, the positive reaction notwithstanding.

Lancilotti-Buonsanti explains the fallacy in tuberculous cattle which have been injected with tuberculin but in which there was no reaction obtained. (1) By the presence of advanced tuberculosis.

(2) By the calcification of the tubercles. (3) The age of the animal below one year. (4) A previous injection of tuberculin during 25 or 30 days preceding the test. (5) The increase in temperature due to extraneous circumstances such as transportation by train immediately preceding the tuberculin test.

The administration of salicylic acid or anti-febrin a few days before the injection of tuberculin is supposed to prevent the reaction, though this has not been proven by any extensive experiments.

THE TUBERCULIN TEST IN ANIMALS THAT HAVE PREVIOUSLY RECEIVED INJECTIONS OF TUBERCULIN.

It has been pointed out by Roeckl, Schuetz and Lydtin that tuberculin tests on animals that have received injections of tuberculin a week prior, that the test was weaker than would normally be expected. Some experiments along this line were also made by Siedamkrotzky and Johne on seven cattle. They came to the conclusion that after repeated injections of tuberculin made at intervals of from four to five days, there is as a rule only a much smaller reaction, and in exceptional solitary cases there is no rise of temperature whatever.

Bartels states that the reaction shortly after a previous initial tuberculin test is not as often absent as is usually believed. He found out of 768 oxen which had given a distinct reaction, that 165 gave a second positive reaction eight days later.

Witt does not entirely coincide with this view of Bartels, as he found out of ten animals which were tested a second time only one gave a positive reaction.

Eber made some similar experiments on 19 cattle which had given a positive reaction on an injection of tuberculin of 0.5 cc. These animals received after 48 hours a second injection of the same dose, and 14 of the 19 animals or 74% gave a positive reaction, while the remaining 5 animals or 26% showed a rise of temperature up to 39.5° C.

According to Nocard, if the tuberculin test is repeated in from 24 to 48 hours following the initial injection, then only one-third of the animals will react; while if applied eight days following the first injection, one-half of the animals will react; and if applied fourteen days following the first injection, two-thirds of the animals will give a positive reaction. It is his belief in order to get a second reaction with the same dose in all cases, an interval of from 25 to 30 days is necessary.

Vallee made a second test after 36 to 48 hours from the initial test, using double the dose of tuberculin injected at the first test. The result was that he obtained in this instance in every animal a second distinct positive reaction.

Stubbe and Mullie report some experiments on 63 cattle which had given a positive reaction following the initial injection of tuberculin. Out of these 63 animals only 37 animals or 56.8% gave a second positive reaction two or three days after the initial test. The quantity of tuberculin used was double the dose injected at the first dose.

Reeser draws from tests made on eight cattle the following conclusions, that as a rule it is possible to obtain several days after the first ordinary tuberculin test a second positive reaction by the injection of a double dose of tuberculin, but this is not always the case. The second reaction sets in quicker than the first one and is somewhat weaker. To obtain a second reaction a larger dose of tuberculin than given at the first test is not absolutely necessary, as one animal which he injected twice with dose of 1 cc. gave a positive reaction each time.

Lueders has published experiments in 284 cattle in which he endeavored to determine whether cattle become exempt to tuberculin. In summing up the work he states that in order to obtain a second positive reaction in all animals which had been previously tested a short time before, it is necessary to use an increased dose of tuberculin. If a dose of 2 cc. of tuberculin is injected at the second test, then it is possible to obtain with certainty a second distinctly positive reaction after an interval of five days from the initial test. If a dose of 2 cc. is used for the second test it is then possible to obtain a positive reaction regardless of whether an ordinary dose of 0.5 cc. or a stronger dose of 1 or 2 cc. of tuberculin was used for the initial test. In order to avoid mistakes he recommends it is necessary when making second tests to give the injection in the early morning hours and to start taking the temperatures immediately after the injection and to continue to do so every two hours until the eighteenth hour. The reactions following the second test are somewhat different from the ones following the first test in that they appear earlier following the injection and are of shorter duration. He claims for the initial test a dose of 0.5 cc. of tuberculin is not sufficient to produce a distinct reaction in all cases, and advises in order to avoid errors in general to employ stronger doses of tuberculin for the initial test of at least 1 cc. for grown cattle.

Kiesig further investigated this question and used 167 cattle, and came to the following conclusions: Cattle which give a thermal reaction at the first tuberculin test will give a second reaction in a large majority of the cases after from eight days to three weeks following the initial test. In a small number a temperature elevation is not obtained. The percentage of animals reacting after a second tuberculin injection depends (1) on the length of interval between the tests, (2) on the dose of tuberculin injected at the time of the first test, and (3) on the dose of tuberculin injected at the time of the second test. As to the length of the interval between the two tests, he found when tuberculous animals were injected after eight days 66.7% of them gave a second reaction. After fourteen days 71.4% gave a reaction and after three weeks 50% gave a reaction. The smaller the dose injected at the first test and the larger the dose injected at the second test, the more certain one was to obtain a distinct second reaction. In tuberculous cattle, if the second test is made from eight days to three weeks following the initial test, the reaction usually occurs in most cases seven hours following the injection of tuberculin. The highest temperature measurements after the second injection are as a rule lower in the same animal than the maximum temperature measurements following the first injection. The dose used for the second injection should be increased to at least 1 cc. and the temperature measurements should be taken every hour from the second hour to the twentieth hour.

Zwick and Titze consider the question of an acquired immunity against tuberculin in cattle a very important matter for the Veterinary profession. They state that now and then attempts are made from dishonest motives to thwart the correct outcome of the tuberculin test. The German Naval Quarantine Station took a great interest in this matter because they were expected since 1897 and up to 1911 to subject to the tuberculin test all cattle imported from abroad, and to reject those showing a positive reaction. The said stations had in force certain regulations concerning the method of applying the test. Before the injection of tuberculin the temperature of the animals was to be taken at least twice, the first time six hours and the second time immediately before the injection. After the injection of tuberculin the temperature was to be taken at least four times, viz., after the 9th, 12th, 15th and 18th hours. The prescribed dose of the undiluted tuberculin was 0.5 cc. for cows and bulls and 0.25 cc. for young animals and 0.1 cc. for calves, this amount to be diluted just before injection with nine times the amount of one-half of 1% Watery Solution of Carbolic Acid. After the last temperature measurements the highest temperature before the injection of tuberculin was to be compared with the highest temperature following the injection, and if the difference amounted to 1.5° C. or more, the animal was then to be considered as suspicious of tuberculosis. In the early years that the German Naval Station carried out this work the results obtained with the tests were correct, but later on they became entirely valueless. The average number of imported animals which gave a positive reaction was about one-half of 1%, while the number of animals found tuberculous at time of slaughtering amounted to 30 or 40%. This flat failure of the tuberculin test was explained by previous treatment of the animals with large amounts of tuberculin. The same authors cite an experiment in which five young animals about eight months of age that had given a positive tuberculin reaction after the first injection were subjected to tuberculin treatment. They first received 0.5 cc. of pure tuberculin and then after four days they received 1 cc. and on the morning of the fifth day 2 cc. of pure tuberculin. On the tenth day they were tested with 0.5 cc. of tuberculin and three of the animals gave a positive reaction, while in two the reaction was doubtful.

VALUE OF TUBERCULIN PREPARED FROM TUBERCLE BACILLI OF THE HUMAN, BOVINE AND AVIAN TYPE.

The question has often arisen whether tuberculin prepared from tubercle bacilli from various sources is better adapted for the tuberculin test of cattle than tuberculin prepared from a mixture of the human and bovine type of the organism. In reviewing the work I find that this question has been investigated by several authors.

Penrose was the first to test both kinds of tuberculin on human beings and he found that bovine tuberculin has qualitatively the same, but quantitatively a considerably more intensive influence on people than tuberculin prepared from tubercle bacilli of the human type.

Kanda has compared bovine tuberculin with human tuberculin in testing cattle for tuberculosis in Japan, and is of the opinion that for the diagnosis of tuberculosis in cattle tuberculin prepared from tubercle bacilli of the bovine type is more suitable and more reliable than that prepared from tubercle bacilli of the human type. He further advocates or recommends the intravenous injection of tuberculin in veterinary practice because the maximum reaction appears earlier following the injection. He found it usually to occur in from six to eight hours.

DeJohne carried on some investigations along this line and made use of tuberculin prepared from tubercle bacilli of the human type as well as tuberculin prepared from tubercle bacilli from goats, cattle, pigs, horses and sheep, and did not observe in his experiments any difference in the action of these different kinds of tuberculin when applied to the testing of cattle. Wollbach, Ernst and Ruppel also report some investigations which agree with those of DeJohne.

Weber and Dieterlen in their diagnostic tests on cattle and guinea pigs could not perceive a difference in action between human and bovine tuberculin as long as the tuberculin was of the same strength. Zwick and Titze have directed their attention in a number of cases to see whether they could perceive a difference in action on cattle between bovine and human tuberculin, and further to note whether the local reaction following the subcutaneous injection is more marked. They report they could never notice any difference and a local reaction was never observed after a subcutaneous injection of either human or bovine tuberculin.

Reeser has carried on some experiments with tuberculin prepared from tubercle bacilli of birds for the purpose of diagnosing tuberculosis in cattle. In his experiments with the bird tuberculin he concludes that with the ordinary dose of 0.35 cc. he was unable to obtain any reaction worth recording. However, he claims a reliable action is obtained if the dose is increased to 1.5 cc. and goes further to say a dose of 2 to 3 cc. gives a violent reaction even in healthy animals. He therefore concludes that tuberculin from the avian tubercle bacilli is useless for diagnosing tuberculosis in cattle.

THE TUBERCULIN TEST IN CATTLE WHICH HAVE BEEN VACCINATED AGAINST TUBERCULOSIS.

It has been found by those interested in the immunization of cattle against tuberculosis, that when the animals were injected with living tubercle bacilli which were non-virulent for cattle, that they became sensitized to tuberculin and would react to the test for an indefinite time like animals that were afflicted with tuberculosis. This fact is a rather important one, for it has bearing upon the inter-state shipments of animals that have been previously immunized.

von Behring states that a distinct tuberculin reaction may be obtained in cattle during the first seven months after the second vaccination, and that this reaction may continue even after it is impossible to demonstrate in the bodies of the immunized animals any tubercle bacilli that were introduced with the Vaccine. He goes further to state that after a certain period of time, which probably is more than a year following the vaccination of the animal, the tuberculin test is invaluable for the purposes of determining whether tuberculous foci are present in the bodies of the animals.

Weber & Titze are of the opinion that the tuberculin test can only be used after vaccination for the purpose of proving the presence of tuberculosis providing three years have passed from the time of vaccination. They used Tauruman as the substance for producing the immunity.

Heymans in his work upon the immunization of cattle showed that when either human or bovine tubercle bacilli were introduced under the skin in Reed Containers, that the animals would react to the tuberculin test in from 15 to 40 days, though after four to six months from the time the tubercle bacilli were placed under the skin, the sensitiveness to tuberculin disappeared. According to investigations of Moussu this over-sensitiveness to tuberculin persists for a year or longer.

THE CUTANEOUS, THE OPHTHALMIC AND THE INTRADERMAL TUBERCULIN TESTS.

Until 1907 only subcutaneous tuberculin test was used for general purposes. However, some investigators had tried the intravenous use of tuberculin with fair results. In that year two new methods were announced for the application of the tuberculin test for the diagnosis of tuberculosis in man. The first consisted in rubbing into a scarified area on the skin a 50% solution of Old Tuberculin and was known as the von Pirquet Cutaneous Test. The second test consisted in the instillation in the conjunctival sac of the eye of a solution of Old Tuberculin and is known as the Calmette and Wolff-Eisner Ophthalmic Test. Both of these tests have given rather favorable results when used in human medicine, but it has been found that the cutaneous test of von Pirquet is not suitable for cattle. The eye test, however, has given better results in the hands of some operators. The test consists in instilling into the conjunctival sac of the eye a few drops of a 50% solution of Old Tuberculin. The lower lid of the eye is held down and the tuberculin dropped between the lid and eye-ball, after which the lids of the eye are rubbed to spread the tuberculin. If after 9 to 18 hours a redness and swelling of the conjunctiva appears, with a purulent or muco-purulent discharge, then the test is considered to be positive and the animal to be afflicted with tuberculosis. Some authors have recommended instilling in the opposite eye a solution of normal salt solution without tuberculin to control the test. There appears to be some difference of opinion among the various authors as to the reliability of the test, and some have obtained results in which tuberculous cattle do not react to the test, and on autopsy the said animals would exhibit lesions of tuberculosis; and again, on the other hand, animals would react to the test which on autopsy no lesions could be demonstrated. Some authors believed that the difference in results could be explained to a certain extent by the different preparations of tuberculin and recommended that purified tuberculin be used for this test.

Later Moussu and Mantoux announced another test known as the intradermal test, and stated that it was their opinion that the intradermal test equals the subcutaneous test in accuracy. The injection is made in the sub-caudal fold of the skin on the under side of the base of the tail with a hypodermic syringe with a short needle. 0.1 or 0.2 of a cc. is used as a dose. The purified tuberculin is used in a 2% solution consisting of 20 milligrams of tuberculin per cc. pysiological salt solution, and the dose of this solution is between 0.1 and 0.2 of a cc. Haring claims to have had very satisfactory results from the use of this test, and in 291 cattle that reacted to the intradermal test the subcutaneous test was subsequently or simultaneously applied and 282 of the animals reacted to same. He further claims that the intradermal test is unreliable when applied a few days after the subcutaneous test, and strongly recommends solutions of an alcoholic precipitated tuberculin of at least 5% strength. He goes further to state that some tuberculous cattle react locally to the intradermal test, but fail to react thermally to the subcutaneous test, while others which react thermally fail to react locally, and believes that by administering the ophthalmic, intradermal and subcutaneous tests simultaneously, a higher percentage of tuberculous cattle will be detected than if dependence is placed on one test alone. He further claims that the intradermal test is especially adapted to the testing of swine and has proven of value in the routine work of the California Hog Serum Laboratory.

Zwicke & Titze in Kolle & Wassermann Handbuch der Pathogenen Mikroorganismen very ably review the use of tuberculin, and in part state as follows: "The diagnostic value of the subcutaneous tuberculin test is generally recognized. In 92 to 98% of cattle which have given a positive reaction after injection of tuberculin, lesions of tuberculosis can be demonstrated at autopsy provided we are not dealing with cattle which for purposes of immunization have been treated with non-pathogenic tubercle bacilli or tubercle bacilli preparations. If tuberculous cattle do not react to the subcutaneous injection of tuberculin, we are then dealing with one of three possibilities; either the animal is afflicted with advanced tuberculosis, which can be determined with certainty by clinical examination, or it has been given an injection of a big dose of tuberculin shortly preceding the tuberculin test, or else we find at autopsy that the lesion is old, completely calcified and encapsulated, and usually a very small one.

"The reaction to tuberculin indicates only that we have the presence of a tuberculous infection, but it gives us no information concerning the extension of the tuberculous process, the seat or the stage of the disease. This holds true for the subcutaneous as well as for the local tuberculin tests.

"The original pure Tuberculin of Koch is just as valuable when used for diagnostic purposes on domestic animals as the later modifications of this preparation. For these purposes it is of no difference whether the tuberculin is prepared from human or bovine tubercle bacilli.

"The dose of Tuberculin for grown-up cattle is 0.5 to 1.2 cc. of pure tuberculin diluted with about 4 cc. of a ½% carbolic acid solution. For young animals from three months to one year old the dose is ½ and for calves up to three months it is from one-fourth to one-third of the dose for grown-up cattle. For horses the same doses hold good as for cattle. The dose for goats and sheep is 0.05 to 0.15 cc.; for pigs 0.05 to 0.25 cc. and for dogs 0.01 to 0.1 cc. The above-mentioned large doses can be used in cattle without hesitation because of several thousand tests which we made we have never noticed that the health of the tuberculous animals suffered lasting injury on account of the tuberculin injections. Carbolic acid is added only to prevent the tuberculin from deteriorating. In case the diluted tuberculin is to be used immediately the carbolic acid need not be added. As a rule, we use for the diluting fluid sterile distilled water. However, boiled hydrant water answers the purpose just as well. The diluted tuberculin must be used as quickly as possible if it is not in a sterile form. The undiluted tuberculin can be kept with care for years without deterioration. Nevertheless, it is advisable to test the strength of old lots of tuberculin before using.

"We look upon the reaction in the subcutaneous injections of tuberculin as strictly speific. We must consider as not proved the statement that occasional cases of actinomycosis, bothriomycosis, abscesses of the internal organs, helminthiasis and other conditions reacted in the same manner to tuberculin as cases of tuberculosis, because we agree with Hutyra and Marek that in such cases it is hard to eliminate the possibility that somewhere in the bodies of the animals tested there is simultaneously present a tuberculous infection. Nor do we know of any researches which would prove beyond a doubt that such non-specific reactions occur.

"In the case of tuberculin tests reported as misleading in their results, we must consider the rules followed in the interpretation of the respective cases. At the same time the investigation of the carcass at autopsy must be made in such a manner that each individual lymphatic gland is carefully examined."

In compiling a review of this work I have drawn upon the works of the following authors, to whom credit is due:

Dixon, Medical News, Philadelphia, 1889 and 1891.
Dixon, Medical and Surgical Reporter, Philadelphia, 1890.
Trudeau, Med. Record, 22. November, 1890, p. 565. Report of the ultimate results obtained in experimental eye tuberculosis by tuberculin treatment and antitubercular inoculation. Trans. of Assoc. of Americ. Phys., 9, Session. Philadelphia, 1894.
De Schweinitz, Medical News, Dec. 8, 1894.
McFadyean, Jour. Comp. Path. & Therap., June; 1901 and March, 1902.
von Behring, Zeitschr, f. Tiermed., Bd. 6, 321 u. 328, 1902.
von Behring, Berl. tierarztl. Wochenschr., 1902.
McFarland, Proceedings Amer. Med. Assoc. Section on Medicine, Philadelphia, 1897.
Patterson, The Lancet, Oct. 30, 1897.
Thomassen, Recueil de med. vet., 1901, 15. Mai und 15. Sept., und 1903, s. 6.
Neufeld, Deutsche med. Wochenschr., 1903, Nr. 37; 1904, Nr. 18 und 34.
Koch, Schutz, Neufeld, Miessner, Arch. f. wiss. u. prakt. Tierheilk., Bd. 31, 545, 1905 und Zeitschr. f. Hyg., Bd. 51.
von Behring, Berl. Klin. Wochenschr., 1903, Nr. 11.
von Behring, Wien klin. Wochenschr., 1903, Nr. 12.
von Behring, Deutsche med. Wochenschr., 1903.
von Behring, Vortrag, 75. Versammlung deutsch. Naturf. u. Aertzte in Kassel, Sept., 1903.
Marks Berl. tierarztl. Wocheschr., 1903, S. 433; 1905, S. 10 und 45.
Casper, Berl. tierarztl. Wochenschr., 1904, S. 708.
Rossignol & Vallee, Revue de la tuberculose, 1906, p. 217 et 466.
Rossignol & Vallee, Bull. de la soc. med. vet., 1906.
Vallee, Rec. de med. vet., T 83; Bull. de la soc. centrale de med. vet., 1906, p. 407. Ann. de l'inst. Pasteur, 1909.
Vallee, Internat. tierarztl. Kongre B im Haag. 1909.
Vallee & Rossignol, Presse veter., Marz 1906.
Thomassen, Verhandlungen, d. 8. intern. tierarztl. Kongresses in Budapest, Bd. 1, 434, 1905.
Thomassen, Ref. Zeitschr. f. Infektionskrankh. d. Haustiere, Bd. 2, H. 2-3, S. 269.
Weber & Titze, I. Mitteilung. Tuberkulose-Arbeiten a. d. Kais. Ges.-Amte, H. 7; II. Mitteilung, H. 9.
Weber & Titze, Tuberkulose-Arbeiten a. d. Kais. Ges.-Amte., 1910, H. 10.
Regner & Stenstrom, Centralbl. f. Bakt., 1. Abt., Ref., Bd. 48, 628, 1909.
Nowak, Zeitschr. f. Infektionskrankh., parasitare Krankheiten und Hygiene der Haustiere, Bd. 6.
Dammann, Arch. f. wiss. u. prakt. Tierheilk., Bd. 34, 345, 1908 und Bd. 37.
Dammann, Verhandl. der 34 und 35. Plenarversammiung des Deutschen Landwirtschaftsrats, 1906 u. 1907.
Ebeling, Berl. tierarztl, Wochenschr., 1905, S. 1.
von Behring, Heft 8 der Beitrage zur experim. Therapie, Berlin, 1903.
von Behring, Vortrag, gehalten am 16, Marz 1904 in Bonn. Milchzeitung, 1904 S. 503.
von Behring, Die Therapie der Gegenwart, 1904, S. 1.
von Behring, Beitrage zur Klinik der Tuberkulose, Bd. 3, H. 2.
von Behring, Heft 10 der Beitrage zur experim. Therapie, Berlin 1905.
von Behring, Verhandl. der. 34 und 35. Plenarversamml. d. deutsch. Landwirtschaftsrats, 1906 und 1907.
von Behring, Tuberculosis, 1906, p. 342.
von Behring, Deutsche Revue, Nov., 1906, Bd. 31, Nr. 2, S. 19 und Nr. 4, S. 145, u. 232.
von Behring, Behringwerk-Mitteilungen, H. 1 und 2.
von Behring, Nobel-Vorlesung, Stockholm, 12, Dez. 1901.
von Behring, Verhandl. d. 35. Plenarversamml. des Deutschen Landwirtschaftrats, 14, Marz 1907.
von Behring, Therapie der Gegenwart, April. 1907, S. 145.
Romer, Beitrage zur exper. Therapie, 1902, H. 5; 1903, H. 6; 1904, H. 7.
Romer, Tuberculosis, Bd. 3, 166, 1904 und 1910
Romer, Beitrage zur Klinik der Tuberkulose, Bde. 4, 9, 13, 17,
Romer, 8. Internat. tierarztl. Kongress in Budapest, Bd. 1, 406, 1905.
Romer, Verhandl. d. Landwirtschaftskammer f. die. Provinz Ostpreu. ssen, 24. Jan. 1906.
Romer, Landwirtschaftl. Centralbl., Amtsblatt d. Landwirtsch.—Kammer f. die Provinz Posen, 1909, Nr. 8-13.
Romer, Berl. klin. Wochenschr., 1909.
Romer, Zeitschr. f. Infektionskrankh., paras. Krankh. u. Hyg. der Haustiere. Bd. 6.

Romer, Kraus & Levaditi, Handbuch der Technik u Methodik der Immunitatsf., Bd. 1, 2. Lief., S. 1063, 1908.

Lorenz, Deutsche tierarztl. Wochenschr., 1903, S. 450.

Lorenz, Berl. tierarztl, Wochenschr., 1903, Nr. 48.

Lorenz, VIII. internat. tierartzl. Kongress in Budapest, 1905.

Lorenz, Zeitschr. f. Tiermed., Bd. 9, S. 1. 1905.

Degive, Stubbe, Mullie, Lienaux, Ann. d. med. vet., 1906, p. 76.

Streliuger, Zeitschr. f. Tiermed., Bd. 10, 118, 1906.

Strelinger, Berl. tierarztl. Wochenscher., 1908, S. 385.

Kern, Berl. tierarztl. Wochenschr., 1908, S. 578.

Weber, Titze & Jorn, Tub.-Arb. a. d. Kais. Ges.-Amte, 1910, H. 10.

Smith, Journ. of Americ. med. association, 28, April, 1906.

Smith, Journ. of med. research, Vol. 18, Nr. 3, 1908.

Haring, Proceedings Amer. Vet. Med. Assoc. 1910.

Grancher & Martin, Semaine med., 1890, Nr. 37 et Bull. med., Annee 4, p. 777, Paris, 1890.

Grancher & Martin, Congres pour l'etude de la tuberkulose, 1891, p. 10.

Grancher & Martin, Revue de la tuberkulose, T. 1, 289, 1893.

Hericourt & Richet, Compt. rend. de l'acad. des sc., T. 115, 842, 1892.

Hericourt & Richet, Compt. rend. de la soc. de Biol., 1890, p. 627.

Hericourt & Richet, Etudes esper. et. clin. sur la tuberkulose, fasc. 2, p. 365, 1892.

Hericourt & Richet, Comp. rend. de l'acad. de sc. T. 114, 854 et 1389, 1892.

Hericourt & Richet, Bull. med., 1892, p. 741 et 906.

Eber, Berl. tierarztl. Wochenschr., 1904, S. 888.

Eber, Zeitschr. f. Tiermed., Bd. 9, 81, 1905.

Eber, Deutsche tierarztl. Wochenschr., 1905, 13. Jahrg.

Eber, Deutsche tierarztl. Wochenschr., 1907, Nr. 40.

Eber, Centralbl. f. Bakt., 1. Abt., Ref., Bd. 40, 545 u. 631, 1907.

Eber, Centralbl. f. Bakt., 1. Abt., Orig., Bd. 44, 463 u. 569, 1907 und Munch. med. Wochenschr., 1907, S. 1705.

Eber, Vortrag bei der 79. Versamml. Deutscher Naturf. u. Aerzte in Dresden, 1907.

Eber, Deutsche tierztl. Wochenschr., 1908, Nr. 23, S. 333.

Eber, Centralbl. f. Bakt., 1. Abt., Ref., Bd. 42, 257, 1908.

Eber, Centralbl. f. Bakt., 1. Abt., Ref., Bd. 44, Nr. 13-14.

Eber, Berl. tierarztl. Wochenschr., 1909, Nr. 29, S. 543.

Eber, Bericht f. d. 9. internat. tierartlz. Kongress im Haag, 1909.

Eber, Berl. tierarztl, Wochenschr., 1909, Nr. 36, S. 666.

Lignicres, Bull. de la soc. centr. de med., oct. 1905, p. 493.

Lignieres, Internat. Vet.-Kongress, Budapest, 1905.

Lingieres, Bull. de la soc. vet. pratique, Mai 1906. Ref. Rec. d. med. vet., 1906.

Lignieres, Bull. de la societe centr. de med. vet., 1906, p. 403.

Lignieres, Bull. de la societe centre. de med. vet., 30 oct., 1907, p. 488.

Lignieres, Recueil de med. vet., 28 fevr. 1907, p. 90 et 112.

Heymans, Bull. de l'acad. royale de med. de Belgique, 1904, p. 780.

Heymans, Arch. internat. de pharm. et de therap., T. 14, 171, 1905.

Heymans, Bull. de l'acad. royale de med. de Belgique, T. 20, 352, 28, April, 1906.

Heymans, Arch. internat. de pharm. et de therap., T. 17, fasc. ½, 1907 et T. 18, p. 179, 1908.

Heymans, Wien, klin. Wochenschr., 1908, S. 893.

Heymans, IX. Internat. Tierarztl. Kongress im Haag, 1908.

Edelmann, Bericht uber das Veterinarwesen im Konigreich Sachsen fur das Jahr 1909.

Levy, Blumenthal & Marxer, Centralbl. f. Bakt., 1. Abt., Orig., Bd. 46, 278, 1908 und Bd. 47, 289, 1908.

Weber, Schutz, Titze & Holland, Tuberkulose-Arbeiten a. d. Kais. Ges.—Amte, 1908, H. 9.

Titze, Verhandlungen des 14. intern. Kongresses f. Hyg. u. Demographie, Berlin, 1907, Bd. 4, 86.

Titze, Tuberkulose-Arbeiten a. d. Kais. Ges-Amte, 1908, H. 9.

Pearson, Leonard, Second annual report of the Henry Phipps Institute for the study, treatment and prevention of tuberculosis, 1906, p. 311 and The veter. journ., 1907.

Pearson, VIII, internat. tierartzl. Kongress in Budapest, Bd. 3, 98, 1905.

Miessner, Berl. tierarztl. Wochenschr., 1907, S. 37.

Miessner, Landwirtschaftl, Zentralbl., Amtsbl. d. Landwirtsch.-Kammer f. d. Provinz, Posen, 1909, Nr. 17, 18 und 19.

Marx, Berl. tierartzl. Wochenschr., 1904 und 1905.

Freidmann, Deutsche med. Wochenschr., 1903, Nr. 50.

Friedmann, Deutsche med. Wochenschr., 1904, Nr. 5.

Friedmann, Deutsche med. Wochenschr., 1904, Nr. 46.

Friedmann, Therapeutische Monatshefte, 1904, S. 123.

Calmette & Gueriu, Compt rend. de l'acad. d. sc., 14. Mai und 11. Juni 1906.

Calmette & Guerin, Ann. de l'inst. Pasteur, 1907, p. 525; 1908, p. 689.

von Behring, Romer & Ruppel, Tuberculose, H. 5 der Beitrage zur experim. Therapie, Marburg, 1902.

von Baumgarten, Berl. klin. Wochenschr., 1904, Nr. 43 und 1905.—Verhandl. d. Deutsch. patholog. Ges. Breslau, 18.—21. Sept. 1904.—Verhandl. d. Deutsch. patholog. Ges. zu Stuttgart, 17.—21. Sept., 1904.
von Baumgarten & Dibbelt, Experimentelle Untersuchungen. III. Bericht. Arb., a. d. pathol. Institut Tubingen, Bd. 6, 52, 1907.
von Baumgarten & Hegler, Berl. klin. Wochenschr., 1905, Nr. 3 und Arb. a. d. pathol. Institut Tubingen, Bd. 5, H. 2, 1905.
von Baumgarten & Kappis, Arb. a. d. Gebiet d. path. Anatomie u. Bakt. a. d. pathol.—anat. Institut Tubingen, Bd. 5, 355, 1906.
Arloing & Stazzi, Ref. in Fortschr. der Veterinarbyg., 1906, S. 190.
Arloing VIII. internat. tierarztl. Kongress in in Budapest, Bd. 3, 101, 1905.
Arloing, Congres intern. de la Tuberculose Paris, T. 1, 216, 1905.
Arloing, Acad. de sciences, 18. Juni 1906.
Arloing, Journ. de Lyon, 1891.
Arloing, Rev. vet., 1891.
Arloing, Soc. de Biologie, 1907, Nr. 27.
von Behring, Berl. klin. Wochenschr., 1899, S. 537.
von Behring, Romer, Ruppel, Beitr. z. exper. Ther., Bd. 5.
Bartels, Deutsche Zeitschr. f. Tiermed., 1895, S. 1.
Bartels, Verwendung des Tuberkulins usw. Leipzig, 1896.
Bartels, Bericht uber 7, intern. tierarztl. Kongr., Bern 1895 und 8, in Budapest, 1905.
Bartels, Deutsche tierarztl. Wochenschr., 1902, S. 285.
Wolff-Eisner, Berl. klin. Wochenschr., 1907, S. 700.
Wolff-Eisner, Beitr. zur Klinik der Tuberkulose, Bd. 9, H. 1.
Wolff-Eisner, Deutsche med. Wochenschr., 1908, S. 180.
Wolff-Eisner, Munch. med. Wochenschr., 1908, S. 65.
Vallee, Ann. de l'inst. Pasteur, T. 9, 1904.
Vallee, Rev. gen. de med. vet., 1904, S. 161.
Vallee, Bull. de la soc. centr. de med. vet., 1907, S. 308.
Sticker, Berl. tierarztl. Wochenschr., 1899, S. 44.
Sticker, Zeitschr. f. Tiermediz., Bd. 19.
Siedamgrotzky & Johne, Bericht uber das Veterinarwesen im Konigreich Sachsen, 1890, S. 101.
Siedamgrotzky, Bericht uber das Veterinarwesen in Konigreich Sachsen, 1891, S. 232.
Freidberger & Frohner, Lehrbuch der spez. Pathologie.
Freidberger & Frohner, Klin. Untersuchungsmethoden.
Eber, Veterinarber. f. das Konigreich Sachsen, 1893. s. 34.
Eber, Deutsche, Zeitschr. f. Tiermed., Bd. 21, 69, 1895.
Eber, Deutsche, tierarztl. Wochenschr., 1895, S. 223.
Eber, Tuberkulinprobe usw. Berlin, Parey, 1898.
Eber, Veterinarkongress Budapest, 1905.
Guerin & Delattre, Opthalmoreaktion. Bull. de la soc. centr. de med. vet., 1907, p. 375.
Hess, Landwirtschaftl. Jahrbuch der Schweiz, 1894, S. 394.
Hess, 6 Veterinarkongress Bern, 1895.
Gutmann, Baltische Wochenschr. f. Landwirtschaft, 1890, Nr. 51; Monatsh f. Tierheilk, 1895.
Hafner & Lydtin, Badische tierarztl. Mitteil., 1891, Nr. 8.
Hahn, Berl. klin. Wochenschr., 1891, Nr. 30; Munch. med. Wochenschr., 1897, Nr. 48.
Hutyra & Marek, Spez. Pathologie der Haustiere.
Malm, VIII. internat. tierarztl. Kongress, Budapest, 1905.
Klimmer & Wolff-Eisner, Handbuch der Serumtherapie, 1911.
Kitt, Wochenschr. f. Tierheilk. u. Viehzucht, 1891.
Kitt, Jahresber, der tierarztl. Hochschule Munchen, 1895.
Ivo & Claude, Opthalmoreaktion. Rec. de med. vet., 1907, p. 507.
Donitz, Klin. Jahrb., Bd. 7, 1908.
Donitz, Handbuch der pathogenen Mikroorganismen von Kolle Wasserman, 1 Aufl., Bd. 4.
Gratz, Opthalmo-und Kutireaktion. Berl. tierarztl. Wochenschr., 1908, Nr. 47.
Frohner, Monatsh. f. prakt. Tierheilk., Bd. 5, H. 2 u. 5.
Garth, Kranich & Grunert, Opthalmo- und Kutanreaktion. Deutsche tierarztl. Wochenschr., 1908.
Gmeiner, Monatsh. f. Tierheilk., Bd. 6, S. 404.
Calmette, Ophthalmoreaktion. Compt. rend. de l'acad. des sc., 1907, 17. Juni.
Romer, Handbuch von Kraus & levaditi, 1908.
Romer & Joseph, Beitr. zur Klinik der Tuberkulose, Bd. 14, S. 1, 1909.
De Schweinitz & Dorset, Centralbl. f. Bakt., 1. Abt., Ref., Bd. 19 und 33.
Rievel, Berl. tierarztl. Wochenschr., 1893, S. 451.
Nocard, Ann. de l'inst. Pasteur, 1892.
Nocard, Deutsche Zeitschr. f. Tiermed., Bd. 22, 1895.
Nocard, Bull. de la soc. centr. de med. vet., 1897, p. 55.
von Pirquet, Berl. med. Gesellschaft, Sitz. vom. 8. v. 1907.
von Pirquet, Berl. klin. Wochenschr., 1907, Nr. 20 und 22.

von Pirquet, Handbuch von Kraus & Levaditi, 1908.
Moussu & Mantoux, Rev. de med. veter., 1908, p. 500.
Neufeld, Deutsche med. Wochenschr., 1900, Nr. 15.
Witt, Berl. tierarztl. Wochenschr., 1904, S. 530.
Schmidt, Maanedsskr. f. Dyrlaege, Bd. 3, 129.
Schumann, Berl. tierarztl. Wochenschr., 1892.
Reeser, Centralbl. f. Bakt., 1. Abt., Ref., Bd. 46, 163.
Mossu, Bull. de là soc. centr. de med. vet., 1907, p. 373.
Kunkel, Berl. klin. Wochenschr, 1892.
Foth, Ophthalmoreaktion. Zeitschr. f. Tiermed., 1908, S. 321 und Berl. tierarztl. Wochenschr., 1909, S. 727.
Degive, Denert, Stubbe, Ann. de Brux., 1892.
Dammann & Mussemeier, Hannover, Schaper, 1905.
Lothes, Berl. tierarztl. Wochenschr., 1891, S. 99.
Luders, Inaug.-Diss., Leipzig, 1908.
Lydtin, Berl. tierarztl. Wochenschr., 1891.
Malkmus, Monatsh. f. prakt. Tierheilk., 1892, S. 164.
Marek, Lehrbuch der klinischen Diagnostik, 1912.
Jensen, Maanedsskr. f. dyrlaeger, 1892-93.
Reinecke, Berl. tierarztl. Wochenschr., 1908, S. 313.
Fenner, Monatsh. f. Tierheilk., 1892, S. 254.
Bang, Berl. tierarztl. Wochenschr., 1891, Nr. 15 u. 16.
Bang, Die Verwendung des Tuberkulins, etc., Leipzig, 1896.
Vanderheyden, Ann. de med. vet., 1907, S. 611.
Schroeder & Mohler, Bureau of Animal Industry, Bull. Nr. 88.
Kiessig, Inaug.-Dissert. Leipzig, 1908.
Kickhaeffer, Berl. tierarztl. Wochenschr., 1892, Nr. 15.
Hutyra, Monatsh. f. Tierheilk., 1891, S. 385.
Hutyra, Zeitschr, f. Tiermed., 1900, S. 31.
Gensert, Berl. tierarztl. Wochenschr., 1894, S. 293.
Stubbe & Mullie, Ann de med. vet., 1905, S. 198.
Zwicke & Titze, Handbuch der pathogenen mikroorganismen Kolle & Wassermann, Band V.
Eber, Veterinarber, f. das, Konigreich, Sachsen, 1893, S. 34.
Eber, Deutsche Zeitschr. f. Tiermed., Bd. 21, 69, 1895.
Eber, Deutsche tierarztl. Wochenschr., 1895, S. 223.
Eber, Tuberkulinprobe usw. Berlin, Parey, 1898.
Eber, Veterinarkongress Budapest, 1905.